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13. SUPPLEMENTARY NOTES					
14. ABSTRACT The aforementioned grant supported a workshop on gas channels highlighting the work related to Gas Channels in Walter Boron's Laboratory. In addition several faculty from other institutions were invited speakers at the workshop.					
15. SUBJECT TERMS Gas channels, aquaporins, Rh proteins, red blood cells, mass spectroscopy, modular dynamics, carbon dioxide (CO2), ammonia (NH3), surface-pH measurements. Xenopus oocytes.					
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I. Heading

- A. PI Name: Walter F. Boron M.D, Ph.D.**
- B. Organization: Case Western Reserve University (CWRU)**
- C. ONR Award Number: N00014-12-1-0646**
- D. Award Title: Gas Channel Workshop**

II. Scientific and Technical Objectives

The grant has two main objectives:

- AIM 1:** CONVENE A GAS-CHANNEL WORKSHOP. Topics to be discussed on Day 1 include: (A) Factors limiting gas permeable through the membrane's lipid phase. (B) Molecular mechanism of gas permeation through AQPs and Rh proteins. (C) Physiological significance of gas channels. (D) O₂ channels. (E) Relevant technologies, including electrophysiology, stopped-flow techniques, mass spectrometry, x-ray crystallography, molecular dynamics and other modeling approaches. Day 2 will be devoted to assessing options for future work.
- AIM 2:** GENERATE A REPORT OF THE MAJOR CONCLUSIONS OF THE WORKSHOP. We will record the presentations (audio, video, as well as content of projector electronic whiteboard) and generate a written summary of the entire Workshop. We will also generate parallel multimedia presentations on our Website as well as in an iPad Textbook format.

III. Approach (N/A)

IV. Concise Accomplishments

We arranged a 2-day meeting—Thursday September 6 through Friday September 7, 2012—held in the Department of Physiology and Biophysics at Case Western Reserve University in Cleveland, Ohio. We hosted 7 outside speakers plus the PI, 3 postdoctoral participants from CWRU, and two additional attendees (see “[Gas Channels Workshop Participants](#)” in Appendix). On the evening preceding the Workshop, we held a welcoming reception at the PI’s home (funded privately). In addition, we held a dinner on Thursday evening (funded privately). Finally, several participants remained for an additional day for scientific discussions.

Aim 1 (Convene a Gas-Channel Workshop). On the first day of the Workshop (see “[Gas Channels Workshop Agenda](#)” in Appendix), the faculty participants made presentations on a range of carefully selected topics. These included a keynote lecture by Robert Stroud at 4PM that was attended by about 160. The other lectures were attended by 50-100. On the second day, three postdoctoral fellows made shorter presentations. In addition, we held a wide-ranging discussion in which we addressed specific questions on the future of gas-channel research.

Aim 2 (Generate a Report). We video-taped the lectures on Day 1 (see videos posted on the Website, described below), and recorded the more informal proceedings of Day 2. The videos are being edited and will be posted on the Website in the near future. In addition, we took notes of all Workshop activities, including the individual presentations (see “[Workshop Notes on Presentations--Rossana](#)” and “[Workshop Notes on Presentations--Walter](#)” in Appendix) and the General Discussion during the latter part of Day 2 (see “[Workshop Notes--General Discussion](#)” in Appendix). After the Workshop, we collected the presentations of all but one of the presenters. Finally, we created a site on the PI’s departmental Website (see <http://physiology.case.edu/events/symposia/gas-channels-workshop-2012/>), where we present the agenda, participants, notes, and links to the presentations. This site is available to all interested parties and will remain live indefinitely.

The consensus among the invited attendees as well as Clevelanders was that Workshop was a great success—in terms of the quality of the participants, the presentations, and the discussions that surrounded the formal presentations. Moreover, the participants strongly indicated that it would be most helpful for the group to get together regularly, perhaps next in 2014.

V. Expanded Accomplishments (N/A)

VI. Work Plan (N/A)

VII. Major Problems/Issues

The only negative aspect regarding the Workshop is that final permission to go forward came relatively late. Although we had tentative commitments from the participants, a firm commitment obviously depended upon the dates chosen for the Workshop and the calendars of the participants remaining open. Immediately upon getting approval for the Workshop, the ONR and the organizers at CWRU did an outstanding job to assemble a first-rate meeting. On the other hand, we could have attracted a few more outstanding speakers as well as representatives of other funding agencies if we had been able to give them more notice. Thus, I recommend that—if we do go ahead with a follow-up meeting, say in 2014—we announce the meeting 8-12 months in advance.

VIII. Technology Transfer

None.

IX. Foreign Collaborations and Supported Foreign Nationals

Two of the meeting participants, Gerolf Gros and Volker Endeward, are from Hannover in Germany.

X. Productivity (N/A)

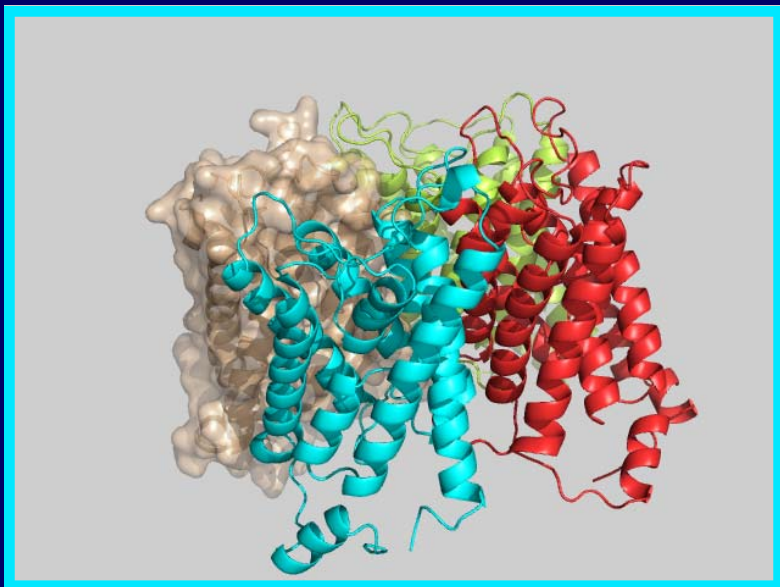
XI. Award Participants

Military personnel: None

Walter F. Boron, M.D., Ph.D—PI

For other participants, see [Gas Channels Workshop Participants](#) in the Appendix.

GAS CHANNELS WORKSHOP



September 6 - 7, 2012

*Case Western Reserve University
School of Medicine
Cleveland, Ohio*

*SPONSORED BY
THE OFFICE OF NAVAL RESEARCH*

Gas Channels Workshop

Thursday, September 6 &
Friday, September 7, 2012

*Department of Physiology and Biophysics
Case Western Reserve University
School of Medicine*

*Sponsored by
The Office of Naval Research*

Welcome to the Workshop on Gas Channels, sponsored by the Office of Naval Research and the Department of Physiology and Biophysics in the School of Medicine at Case Western Reserve University.

The ONR handles the science and technology programs of the US Navy and Marine Corps. Divers, submariners, and individuals ascending to altitude may face a range of medical issues related to dissolved (or undissolved) gases. These include decompression illness, N₂ narcosis, O₂ toxicity, CO₂ narcosis, and hypoxia. Thus, the ONR has a strong and longstanding interest in gas transport.

The Department of Physiology and Biophysics at Case Western Reserve University is one of the few in the world that studies physiological problems from the level of the atom—through molecules, cellular organelles, whole cells, tissues, and organs—to the whole organism. We focus on the nervous, cardiovascular, and renal systems. In the past few years, we have recruited eight outstanding new faculty members. In addition, we completed a major renovation of about 40,000 gross square feet of space. We have also established three major core facilities to support our work: A Protein Expression, Purification, and Crystallization Core (PEPCC, 5th floor), a Molecular Biophysics Core (6th floor), and a Mouse Physiological Phenotyping Core (MPPC, basement).

We hope that you enjoy the Workshop. If during your visit you would like to see our facilities, we would be happy to arrange a tour.



WORKSHOP AGENDA
Thursday, September 6, 2012

7:45 - 8:15am

Registration & Continental Breakfast

8:15am - 8:20am

Welcome/ Introduction

-Walter F. Boron, M.D., Ph.D.

8:20am - 9:00am

Walter Boron, M.D., Ph.D.

Title: "Gas Channels"

9:00am - 9:10am:

Question/Answer Session

9:10am - 9:50am

Emad Tajkhorshid, Ph.D.

Title: "Visualizing gas permeation pathways through proteins at sub-Angstrom resolution"

9:50am - 10:00am:

Question/Answer Session

10:00am - 10:25am:

Morning Break

10:25am - 11:05am

Gerolf Gros, Ph.D.

Title: "Measuring cellular CO₂ permeability by ¹⁸O exchange—methodology and results on red blood cells"

11:05am - 11:15am: *Question/Answer Session*

11:15am - 11:55am

Volker Endeward, Ph.D.

Title: "Intrinsic CO₂ permeability of cell membranes and effect of cholesterol and aquaporin"

11:55am - 12:05pm: *Question/Answer Session*

12:05pm - 1:05pm

Lunch –On your own

(Lunch provided for invited speakers in E-504)

1:05pm - 1:45pm

Bhanu Jena, Ph.D.

Title: “Involvement of elevated membrane cholesterol on G-protein regulated water and gas transport in biological membranes”

1:45pm- 1:55pm: Question/Answer Session

1:55pm - 2:35pm

Jeffrey Garvin, Ph.D.

Title: “NO transport by aquaporin 1”

2:35pm - 2:45pm: Question/Answer Session

2:45pm - 3:10pm: Afternoon Break

3:10pm - 3:50pm

David Weiner, M.D.

*Title: “Role of Rh glycoproteins in gas transport
— lessons from in vitro model systems”*

3:50pm - 4:00pm: Question/Answer Session

4:00pm - 5:00pm

Robert Stroud, Ph.D.

Title: "What do structures of the Aquaporins, and Ammonia transporters tell us about conduction of gases?"

WORKSHOP AGENDA
Friday, September 7, 2012

8:00am - 8:30am

Continental Breakfast

8:30am – 8:35am

Introduction/Welcome

-Dr. Walter Boron, M.D., Ph.D.

8:40am – 9:00am

Speaker: Ryan Geyer, Ph.D.

Title: “Role of membrane proteins in oxygen transport in red blood cells”

9:05am – 9:25am

Speaker: Rossana Occhipinti, Ph.D.

Title: “Mathematical modeling of gas movements in an oocyte ”

9:30am - 9:50am

Speaker: Xue Qin, Ph.D.

Title: “Structure determinants for CO₂ transport of human aquaporin 5”

10:00am – 10:30am

Morning Break

(Refreshments served)

10:30am - 1:00pm

Gas Workshop Meeting

1:00pm - 2:00pm

Lunch

2:00pm - 3:30pm

Gas Workshop Meeting

3:30pm –End of Meeting

Walter F. Boron, M.D., Ph.D.
—Case Western Reserve University
Cleveland, Ohio



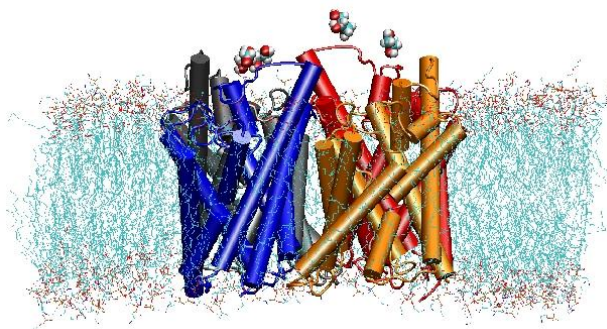
Dr. Boron is the David N. and Inez Myers/ Antonio Scarpa Professor & Chairman of the Department of Physiology and Biophysics at Case Western Reserve University. He earned his AB in chemistry at Saint Louis University, and his M.D. and Ph.D. (Physiology & Biophysics) at Washington University in St. Louis. He joined Yale University as a post-doctoral fellow with Emile Boulpaep in 1978, and remained there for the next 29 years, serving as Chairman of the Department of Cellular & Molecular Physiology for three 3-year terms (1989-1998). In 2007 he returned to his hometown of Cleveland. He is the former President of the American Physiological Society (APS) and is currently Secretary-General of the International Union of Physiological Sciences (IUPS). He is the former editor-in-chief of Physiological Reviews and is the former editor-in-chief of Physiology. He and Emile Boulpaep co-edit the textbook Medical Physiology. He developed his life-long interest in acid-base transport and intracellular-pH regulation with his Ph.D. mentor Albert Roos as well as Paul De Weer, and his complementary interest in renal HCO_3^- transport with Boulpaep. His group currently focuses on three related areas: the molecular physiology of the Na^+ -coupled HCO_3^- transporters, molecular $\text{CO}_2/\text{HCO}_3^-$ sensors, and gas channels. Among his previous honors are a Young Investigator Award (American Society of Nephrology/American Heart Association, 1986), the Robert F. Pitts Award (IUPS, 1993), the Gottschalk Award (APS, 1998), an NIH MERIT Award (2002), the Homer Smith Award (ASN, 2005), the Sharpey-Schafer Award (The Physiological Society, 2008), and the Palade Gold Medal (shared with William Catterall and Richard Tsien, Wayne State University, 2010).

Emad Tajkhorshid, Ph.D.
-University of Illinois at Urbana-Champaign
Urbana, Illinois



Emad Tajkhorshid received his initial training as a pharmacist from Tehran University. After attending two Ph.D. programs, one in medicinal chemistry and pharmacology at Tehran University of Medical Sciences and another one in molecular biophysics at the University of Heidelberg, he started his postdoctoral

training in Computational Biophysics in the Theoretical and Computational Biophysics Group at the University of Illinois at Urbana-Champaign in 2001. In 2003 he became the assistant director of research of the NIH Center for Macromolecular Modeling and Bioinformatics at the Beckman Institute for Advanced Science and Technology. He started his independent career as an assistant professor of biophysics, biochemistry, and pharmacology in 2007 and was promoted to associate professor in 2010. The primary focus of his research is on understanding the structural and dynamical properties of membranes and membrane proteins as a basis for their biological function. Employing computational methodologies, his group investigates a wide range of membrane proteins and membrane-associated phenomena in biological systems, in particular the mechanisms of passive and active transport across the membrane.



Gerolf Gros, M.D., Ph.D.
–Hannover Medical School
Hannover, Germany



Dr. Gros is Professor of Physiology at the Department of Physiology at the Medizinische Hochschule Hannover/Germany. He was Professor and Chairman of this Department from 1986 to 2008. He earned his MD degree in 1969 at the University of Tübingen/Germany, followed by one year of practical clinical work. In 1970 he

joined Hannover Medical School as a postdoc with Waldemar Moll, and joined his mentor when he moved to the Department of Physiology at the University of Regensburg in 1972. Intermittently, he worked at the Department of Physiology with Robert E. Forster in 1973-1974. He obtained his "Habilitation" in Physiology after returning to Regensburg in 1976. From 1978-1986 he was Associate Professor of Physiology at the University of Essen, and thereafter moved to Hannover to become Full Professor and Department Chairman. He was President of the German Physiological Society in 2007, and President of the Annual Congress of Physiology held in Hannover in 2007. He developed a life-long interest in CO_2 and O_2 transport in the body, in carbonic anhydrases and in acid-base physiology, initially stimulated by Waldemar Moll and Robert E. Forster. After his move to Hannover, he developed a second field of interest in studying the molecular mechanisms of skeletal muscle plasticity. His work was continuously supported by the Deutsche Forschungsgemeinschaft. His most recent interest is in the field of CO_2 channels in biological membranes, in combination with developing a novel method to determine the CO_2 permeability of cell membranes, and in the molecular mechanism of HCO_3^- transfer across the red cell membrane.

Volker Endeward, Ph.D.
–Hannover Medical School
Hannover, Germany



Dr. Endeward is presently Asst. Professor of Physiology in the Department of Physiology of the Medizinische Hochschule Hannover/Germany. From 1983-1995 he studied Physics at the University of Hannover and obtained his "Diplom" in 1995. Partly simultaneously, he studied Medicine at the Medizinische Hochschule Hannover from 1986-1995. From 1996 to 1997 he practiced Surgery at the Agnes-Karll hospital in Laatzen/Hannover. In 1998 he joined the Department of Physiology of Hannover Medical School and developed his research interests in CO₂ and O₂ transport and acid-base physiology in Gerolf Gros' laboratory. He has worked and published on several topics in these areas, but his main interest over the last years has been CO₂ channels in biological membranes. He has essentially contributed to the development of the mass spectrometric ¹⁸O exchange technique to measure CO₂ permeabilities of cell and vesicle membranes, including the complex mathematical description of this process and a numerical procedure to derive CO₂ and bicarbonate permeabilities from mass spectrometric measurements. He has further developed this technique by an analysis of the size and role of unstirred layers and by modelling the intracellular processes influencing the process of ¹⁸O exchange. He has presented a comprehensive experimental analysis of the role of aquaporin 1 as a CO₂ channel in the human red cell membrane, as well as the first report that the red cell Rhesus protein RhAG also acts as a CO₂ channel. Most recently he has shown that the intrinsic permeability of many biological membranes is low and identified the molecular cause of this property. In addition, he has presented a comprehensive reinvestigation of the so-called metabolon hypothesis, which proposes the existence of a functionally relevant complex of the anion exchanger 1 and carbonic anhydrase 2 in the red cell membrane. His scientific success was recognized by a special personal grant awarded to him by the Deutsche Forschungsgemeinschaft in 2009.

Bhanu Jena, Ph.D.

**–Wayne State University School of Medicine
Detroit, Michigan**



Dr. Bhanu Jena was born in a small town in Orissa, India, [REDACTED], to Manju Prova and Prafulla K. Jena, a chemist. He spent his early childhood in several remote villages in India, where his grandfather practiced medicine. The dedication of his father and grandfather to science and medicine and their service to humanity greatly influenced his choice

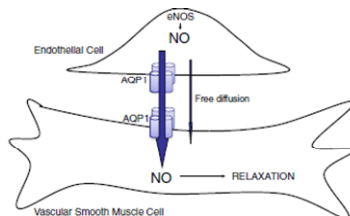
for a career in science. Dr. Jena majored in Chemistry, Zoology and Botany from BJB College in India (B.Sc., 1975) and studied Reproductive Endocrinology at Utkal University, (M.Sc., 1978). He graduated top of his class in the Masters program receiving the Prasant Ku. Memorial Prize and the Utkal University Gold Medal. In December 1988, Dr. Jena received his Ph.D. degree in Reproductive Endocrinology, and the Research Excellence Award from Iowa State University. Following postdoctoral training at Yale University, he joined Yale University as an Assistant Professor. In 2000, Dr. Jena moved to the Department of Physiology, at Wayne State University School of Medicine, as a tenured Professor, and Founder-Director of the Institute of NanoBioScience. His foray into science began 40 years ago, when he published his first scientific paper. His enquiry on how cells secrete, led to the serendipitous discovery of the “porosome” - a new cellular structure universally present in all secretory cells at the cell plasma membrane, and involved in secretion. In early 2012, the neuronal porosome proteome was determined. The current focus of the laboratory is to further determine the structure and conformation of the neuronal porosome using cryo electron crystallography.

Jeffrey Garvin, Ph.D.
-Henry Ford Hospital
Detroit, Michigan



Jeffrey Garvin, Ph.D. is currently Professor of Physiology at Wayne State University and Division Head of the Hypertension and Vascular Research Division of Henry Ford Hospital. He received his B.S. from the University of Miami in Biology and Chemistry in 1979 and his Ph.D. from Duke University in 1984. Dr.

Garvin did his postdoctoral training in the Laboratory of Kidney and Electrolyte Metabolism at the National Institutes of Health under Maurice Burg, Mark Knepper and Kenneth Spring where he was supported by a National Kidney Foundation fellowship and two National Research Service Awards. In 1988 he joined the Hypertension and Vascular Research Division of Henry Ford Hospital and became Division Head in 2009. His research deals with the regulation of transport processes in the kidney and how dysregulation of these systems can contribute to hypertension. Currently he has more than 125 original publications on renal physiology. Dr. Garvin is a fellow of the Council for High Blood Pressure Research of the American Heart Association and has served on several NIH study sections. He also is an Associate Editor of The American Journal of Physiology: Renal Physiology. His research is now supported by three NIH grants, including a Program Project Grant entitled "Blood Pressure Regulation: Novel Roles for the Kidney."



David Weiner, M.D.
-University of Florida
Gainesville, Florida



Dr. Weiner's primary research interests involve examining the mechanisms and regulation of renal ammonia metabolism and transport. Ammonia plays a central role in acid-base homeostasis, as it is the primary component of basal net acid excretion and changes in ammonia excretion comprise almost 90% of the renal response

to acid-base alterations. Renal ammonia transport has traditionally been believed to involve "ammonium (NH_4^+) trapping" and diffusive NH_3 movement.

Dr. Weiner's laboratory examines the specific mechanisms of renal NH_3 movement, and has shown that, in contrast to previously thought models, that NH_3 transport involves specific proteins, namely, Rh glycoproteins. These proteins are widely expressed in ammonia transporting tissues, and Dr. Weiner's studies, using a variety of in vivo and in vitro models, including transgenic animal models utilizing cell-specific gene deletion, have shown the central role of these proteins in renal ammonia, and thereby acid-base, homeostasis.



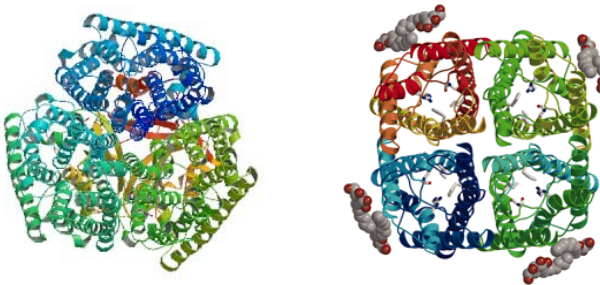
Robert Stroud, Ph.D.
-University of California San Francisco
San Francisco, California



*Dr. Stroud was the first to discover fundamental mechanisms of transmembrane proteins by 'Aquaporins' at atomic resolution. These included GlpF, AqpZ, the eye lens AQP0, the H₂S channel, and the essential glycerol channel of the malaria parasite *P.falciparum*. He defined the structure and regulatory mechanisms of the ammonia channel AmtB and the 'Rh factors'.*

He revealed the atomic basis for 'signal sequence' dependant membrane protein synthesis, signaling by EPO (erythropoietin) via its receptors. Stroud also determined the mechanisms of enzyme drug targets thymidylate synthase, HIV protease, HIV integrase, and KSHV protease and used these to facilitate drug discovery for human health.

He was elected to the National Academy of Sciences (of the USA) in 2003, President of the Biophysical Society (of the United States) from 1986-1987, and Founding Fellow of the Society in 2000. Dr. Stroud is a member of the Committee for the International Union of Pure and Applied Biophysics. In 1984 he was elected the DeWitt Stetten Lecturer of the National Institutes of Health (NIH). Dr. Stroud was elected as a Fellow of the Royal Society of Medicine (United Kingdom) in 1992.



Case Western Reserve University Boron Lab Post-doctorates



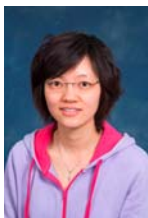
R. Ryan Geyer, Ph.D. is currently a postdoctoral research fellow in the Department of Physiology and Biophysics at Case Western Reserve University. He received his B.A. in biology from Earlham College in 1998 and his Ph.D. in biochemistry and molecular biology from Wright State University in 2007. In 2008,

Dr. Geyer joined Dr. Boron's laboratory and has focused his attention towards elucidating the role of membrane proteins in red blood cell oxygen transport. Dr. Geyer is currently supported by a postdoctoral fellowship from the Office of Naval Research.



Rossana Occhipinti, Ph.D. joined Dr. Boron's laboratory as a postdoctoral fellow in October of 2009 shortly after obtaining her Ph.D. in Applied Mathematics from Case. During her Ph.D. studies she developed mathematical models of cellular brain metabolism and numerical methods combining optimization algorithms with Bayesian statistics. She is currently developing mathematical

models to investigate the movement of acid-base equivalents across the plasma membrane. In 2009, she received the Melvin H. Knisely International Award and in 2012 the Cell & Molecular Physiology Section Research Recognition Award. Her work is currently supported by an AHA Postdoctoral Fellowship.



Xue Qin, Ph.D. earned her Ph.D. in Pathophysiology at Peking University in China. In 2008 she joined Case Western Reserve University in Dr. Boron's lab. Dr. Qin's Ph.D. work was about the signaling pathway of Nitric Oxide, cGMP and Protein Kinase G in coronary arteries. In Dr Boron's Lab, her research has mainly focused on gas channels. Dr. Qin uses surface pH method to study the structural functional relationships of human aquaporin 5.

Her work has been supported by American Heart Association Postdoctoral Fellowship. In 2010 she won the Cell & Molecular Physiology Section Research Recognition Award.



GAS CHANNELS WORKSHOP PARTICIPANTS

Speakers:

Walter F. Boron, MD, PhD

*-Chairman, Professor, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106*

Phone: 216-368-8978

Email: wfb2@case.edu

Volker Endeward, PhD

*-Assistant Professor, University of Hannover, Germany
University of Hannover
Welfengarten 1 Hannover, DE, 30167
Germany*

Phone: 49-511-552711

Email: endeward.volker@mh-hannover.de

Jeffrey Garvin, PhD

*-Professor, Wayne State University/Henry Ford Hospital
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106*

Phone: 216-368-3353

Email: jlg5@case.edu

Gerolf Gros, MD, PhD

*-Professor, University of Hannover, Germany
University of Hannover
Welfengarten 1 Hannover, DE, 30167
Germany*

Phone: 49-577-532-2735

Email: Gros.gerolf@mh-hannover.de

Bhanu Jena, PhD

*-Professor, Wayne State University
Wayne State University
540 Canfield
5245 Gordon Scott Hall
Detroit, MI 48201*

Phone: 313-577-1532

Email: bjena@med.wayne.edu

Robert Stroud, PhD

*-Professor, University of California San Francisco
University of California San Francisco
600 16th Street rm. S414
San Francisco, CA 94107*

Phone: (415) 476-4224

Email: stroud@msg.ucsf.edu

Emad Tajkhorshid, PhD

*-Professor, University of Illinois at Urbana-Champaign
University of Illinois at Urbana-Champaign
Beckman Institute
405 N Mathews Ave
Urbana, IL 61801*

Phone: 217-244-6914

Email: emad@life.illinois.edu

David Weiner, MD

*-Professor, University of Florida
University of Florida
1600 SW Archer Road
Room VA A531, VA Building
PO Box 100224
Gainesville, FL 32610-0277*

Phone: (352) 327-8069

Email: david.weiner@medicine.ufl.edu

Postdoctoral presenters:

Ryan Geyer, PhD

*-Postdoc, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106*

Phone: 216-368-5530

Email: rrg8@case.edu

Rossana Occhipinti, PhD

*-Postdoc, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue*

Cleveland, OH 44106

Phone: 216-368-3631

Email: rxo22@case.edu

Xue Qin, PhD

*-Postdoc, Case Western Reserve University
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106*

Phone: 216-368-6854

Email: xue.qin@case.edu

Additional Attendees:

CDR Matthew Swiergosz, USN

*-Program Manager, Office of Naval Research
Office of Naval Research
Office of Naval Research, Code 342
875 North Randolph Street, Suite 1425
Arlington, VA 22203-1995*

Phone: 703-696-0367

Email: matthew.swiergosz@navy.mil

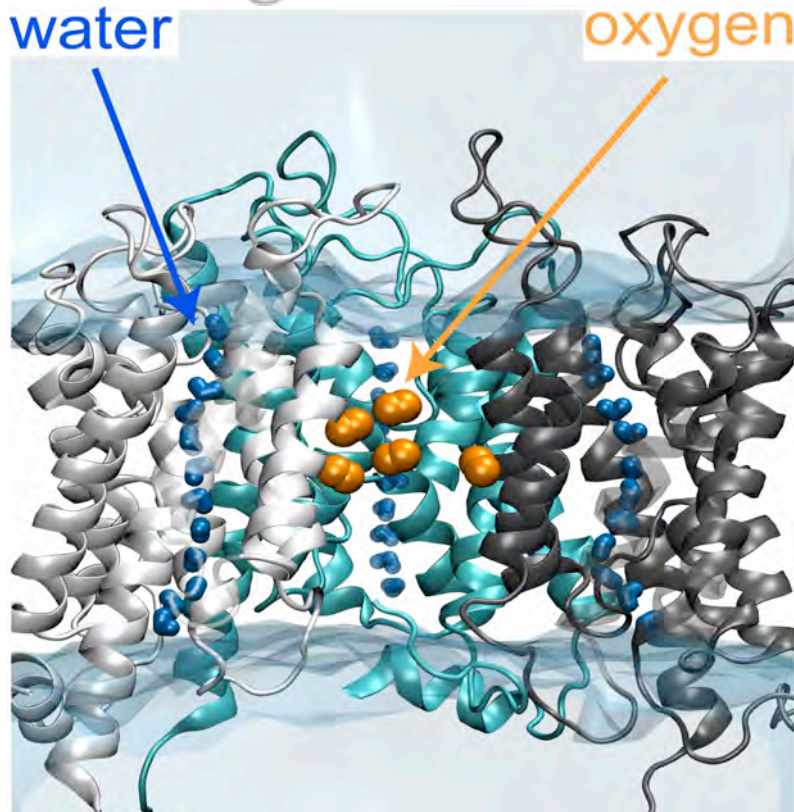
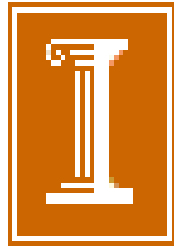
Noah Malmstadt, PhD

*-Assistant Professor, USC
University of Southern California
Mork Family Department of Chemical Engineering and Materials Science
University of Southern California
925 Bloom Walk, HED 216
Los Angeles, CA 90089-1211*

Phone: 213- 821-2034

Email: malmstad@usc.edu

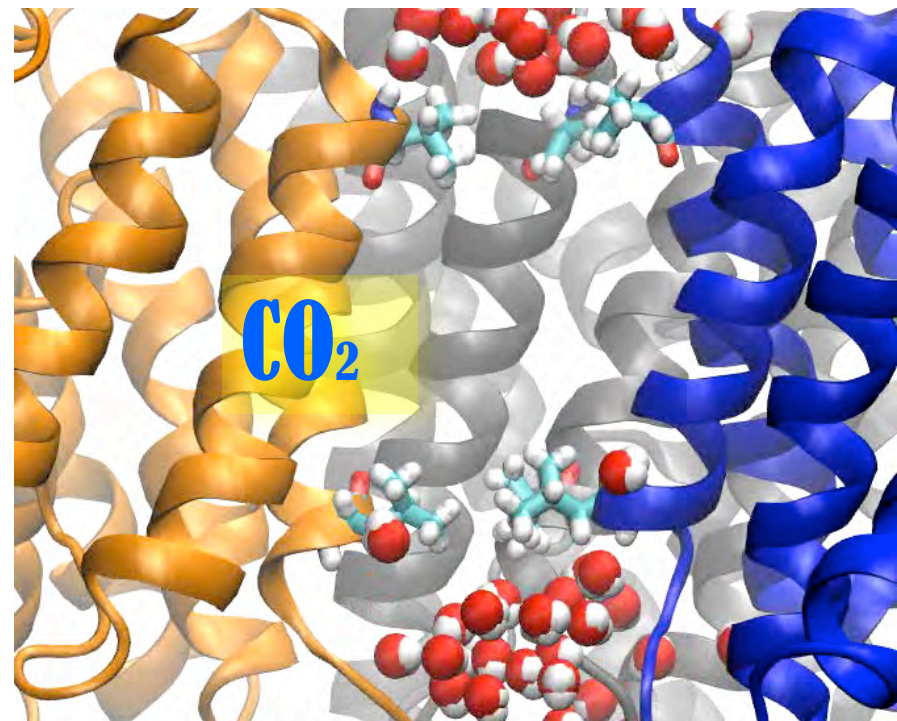
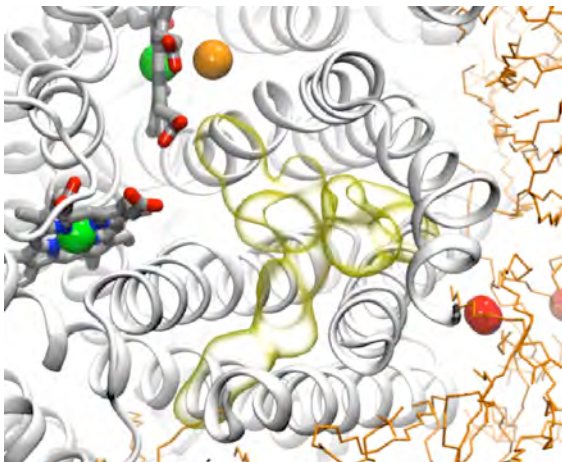
Visualizing Gas Permeation Pathways Through Proteins at Sub-Angstrom Resolution



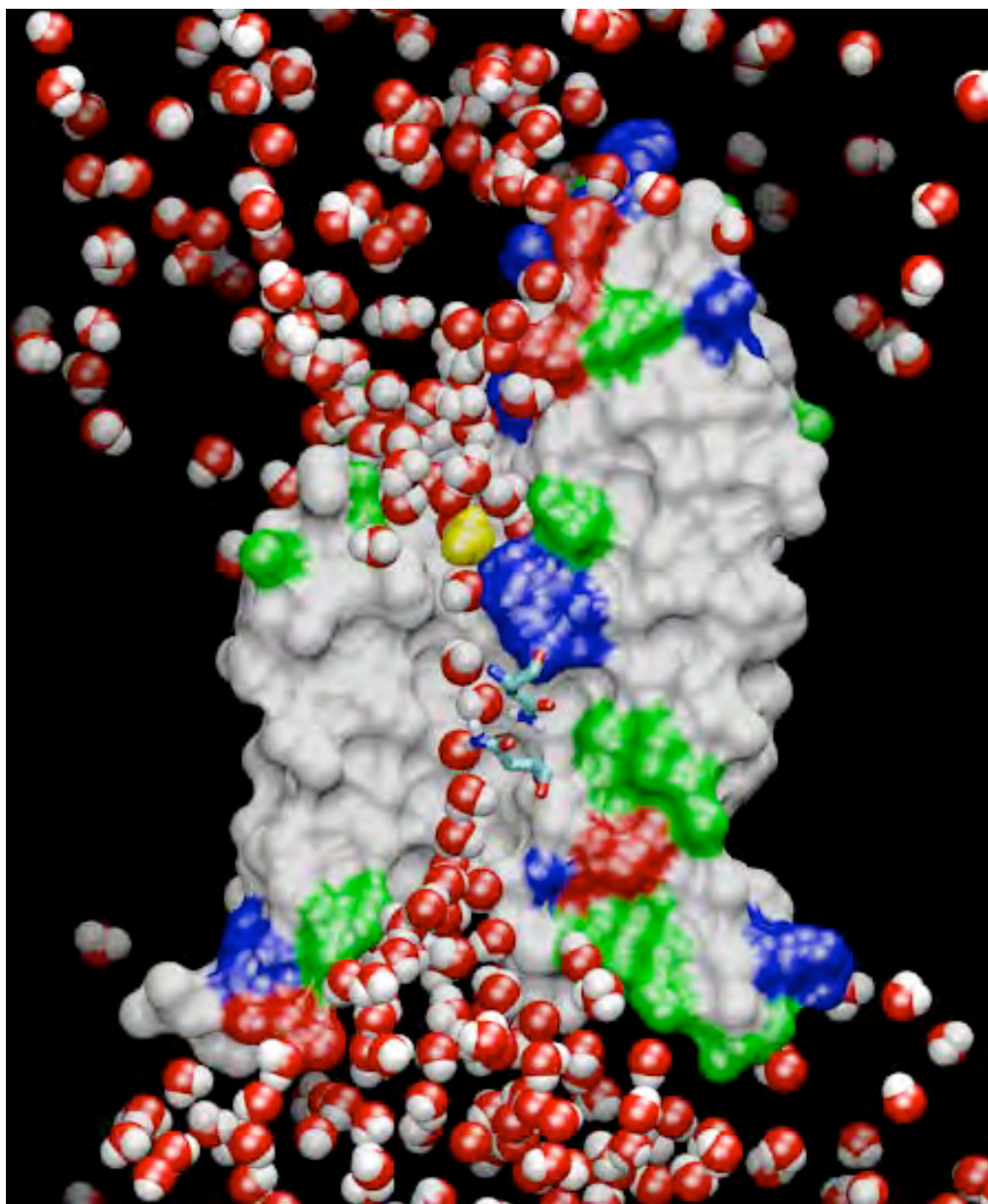
Emad Tajkhorshid

Computational Structural Biology and Molecular Biophysics
www.csbmb.beckman.illinois.edu

Department of Biochemistry
Center for Biophysics and Computational Biology
Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign



Molecular Dynamics Simulations



Solving the Newtonian equations of motion for all particles at every time step

Major limitations:

- Time scale / sampling
- Force field approximations

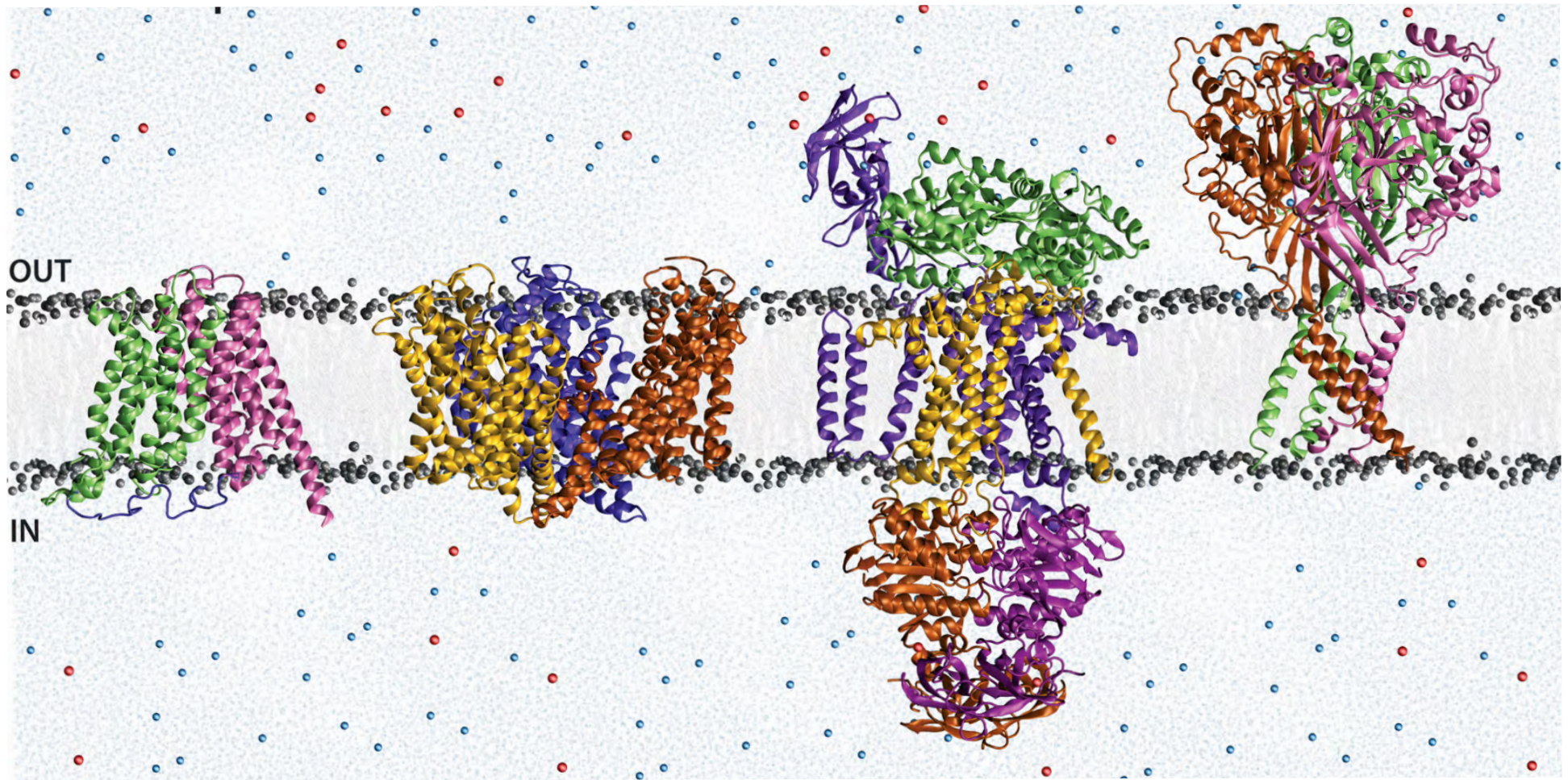
**SPEED
LIMIT**

1 fs

Major advantages:

- Providing a dynamical description
- Unparalleled spatial and temporal resolutions, **simultaneously**

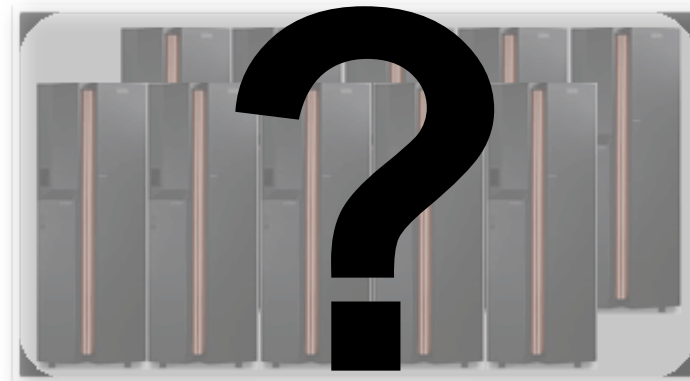
In situ Molecular Dynamics Simulations



Atom count: 100-500k
~10 ns/day on 128-1024 processors
100-500 ns for each system

Fast Growth of Computational Power

HP 735 cluster
12 processors
(1993)



Blue Waters (UIUC)
200,000+ processors (2013)



SGI Origin 2000
128 processors (1997)



PSC LeMieux AlphaServer SC
3000 processors (2002)

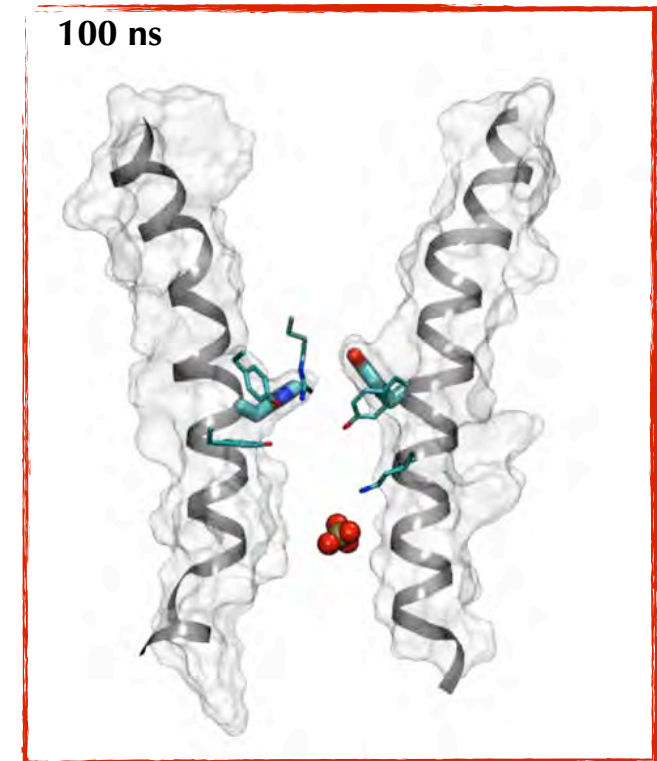
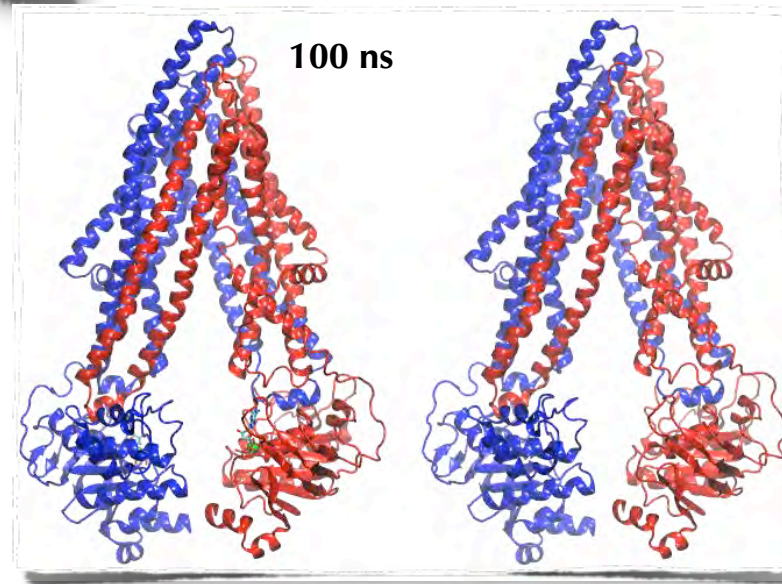
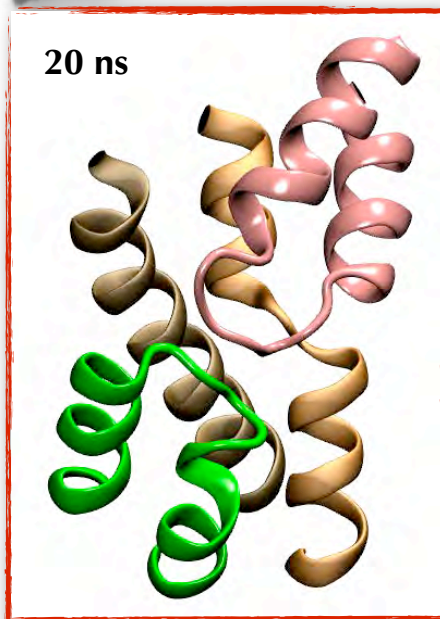
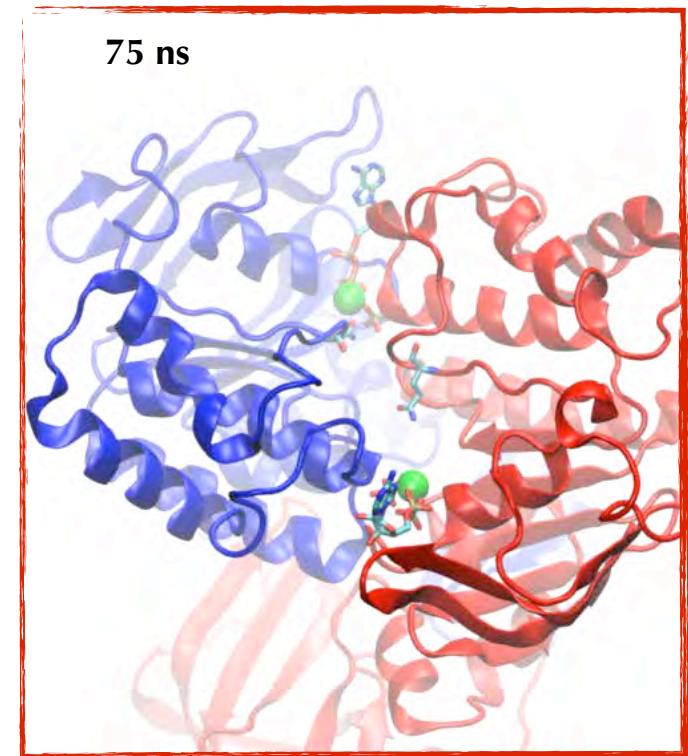
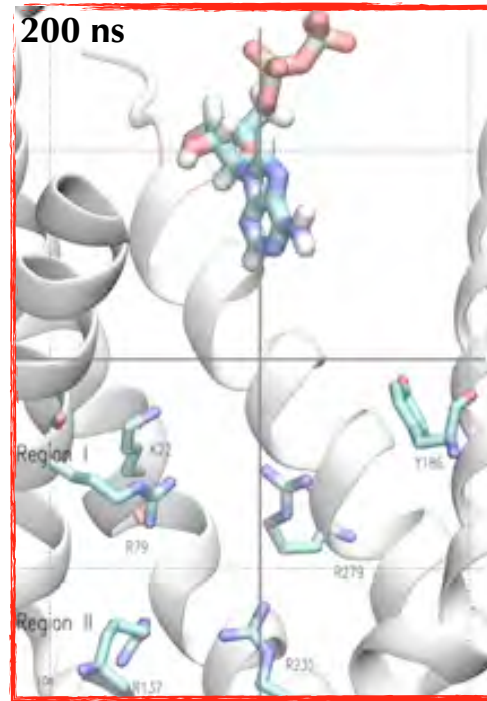
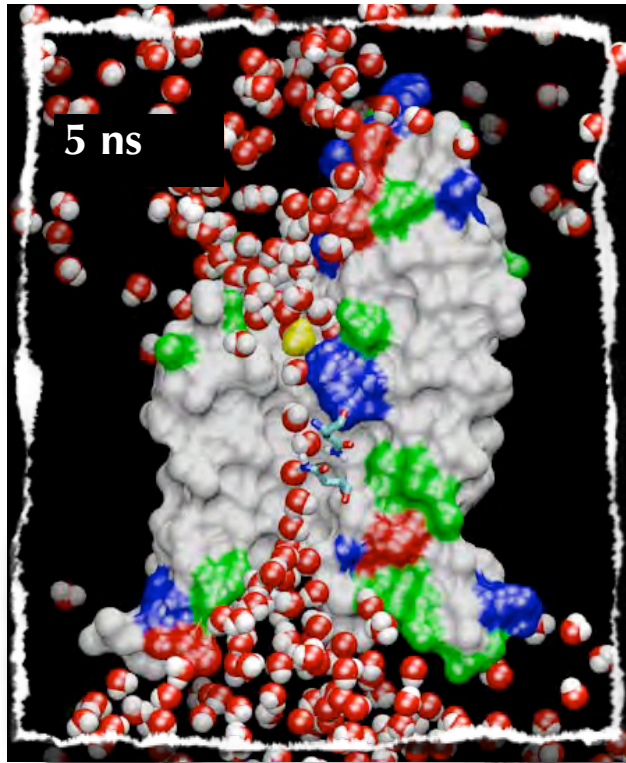


Ranger/Kraken
~60,000 processors (2007)

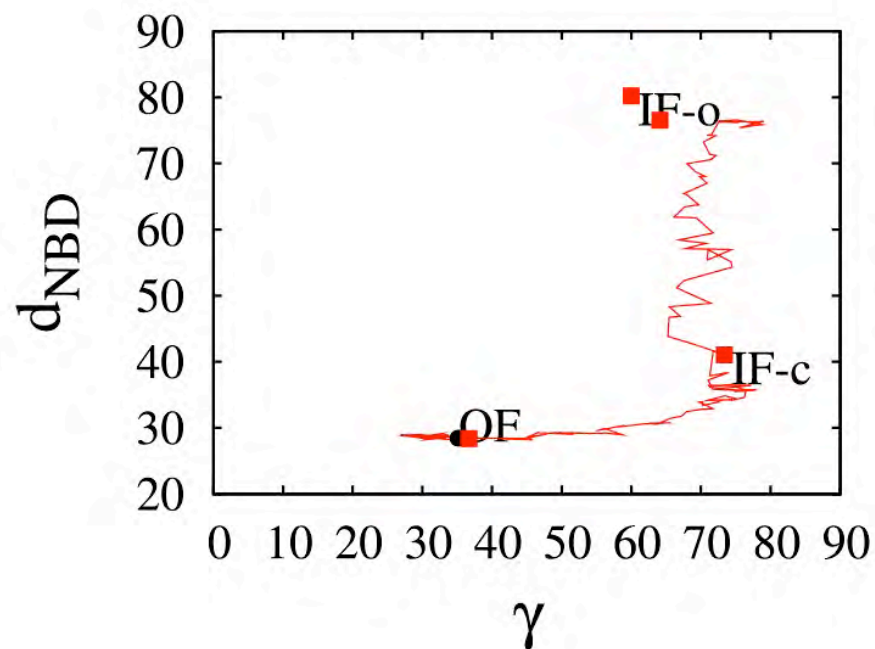
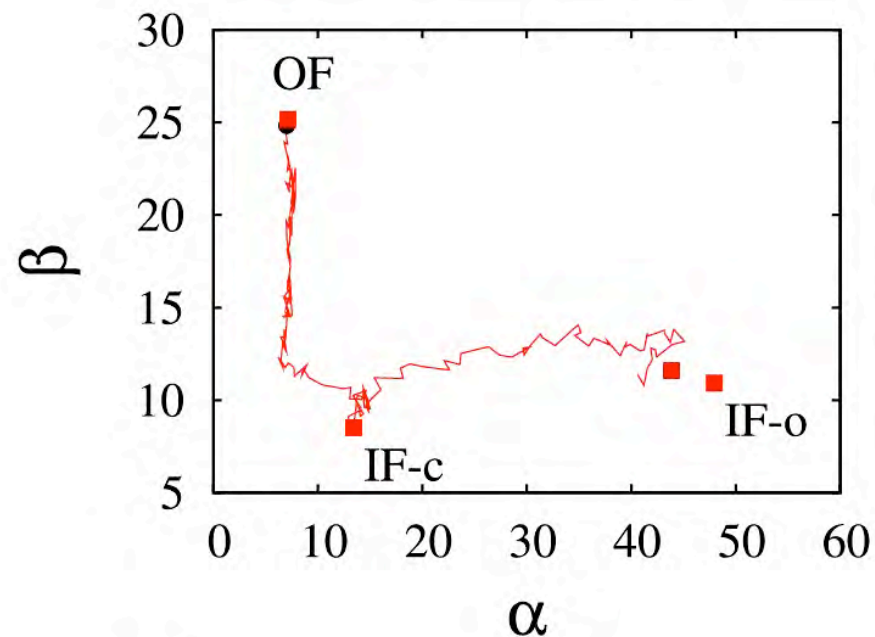
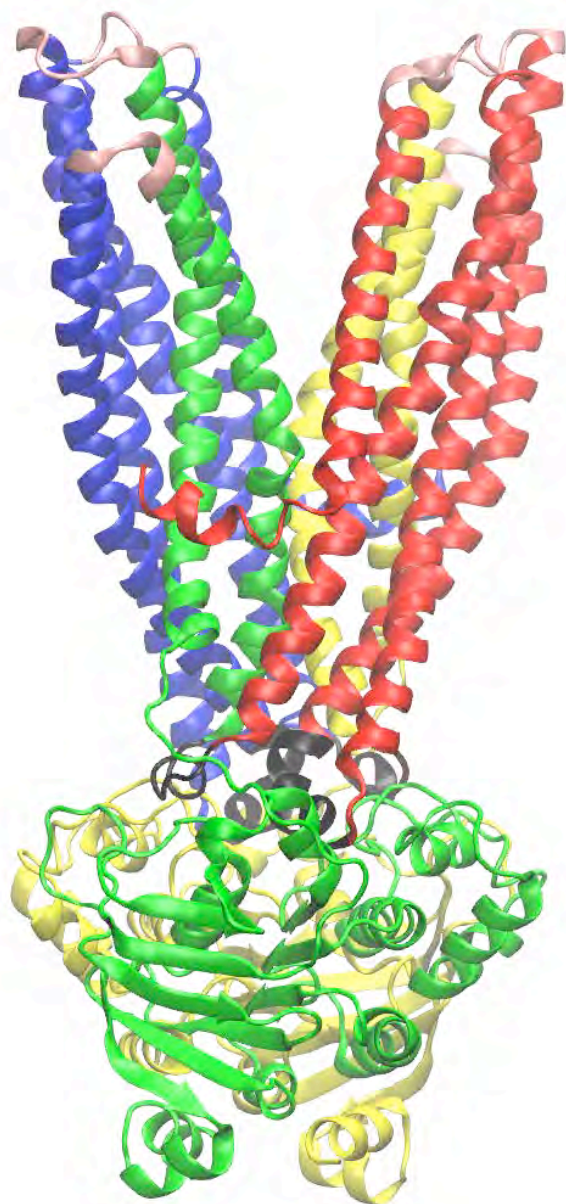


Anton/DESHAW/PSC
512 processors (2010)

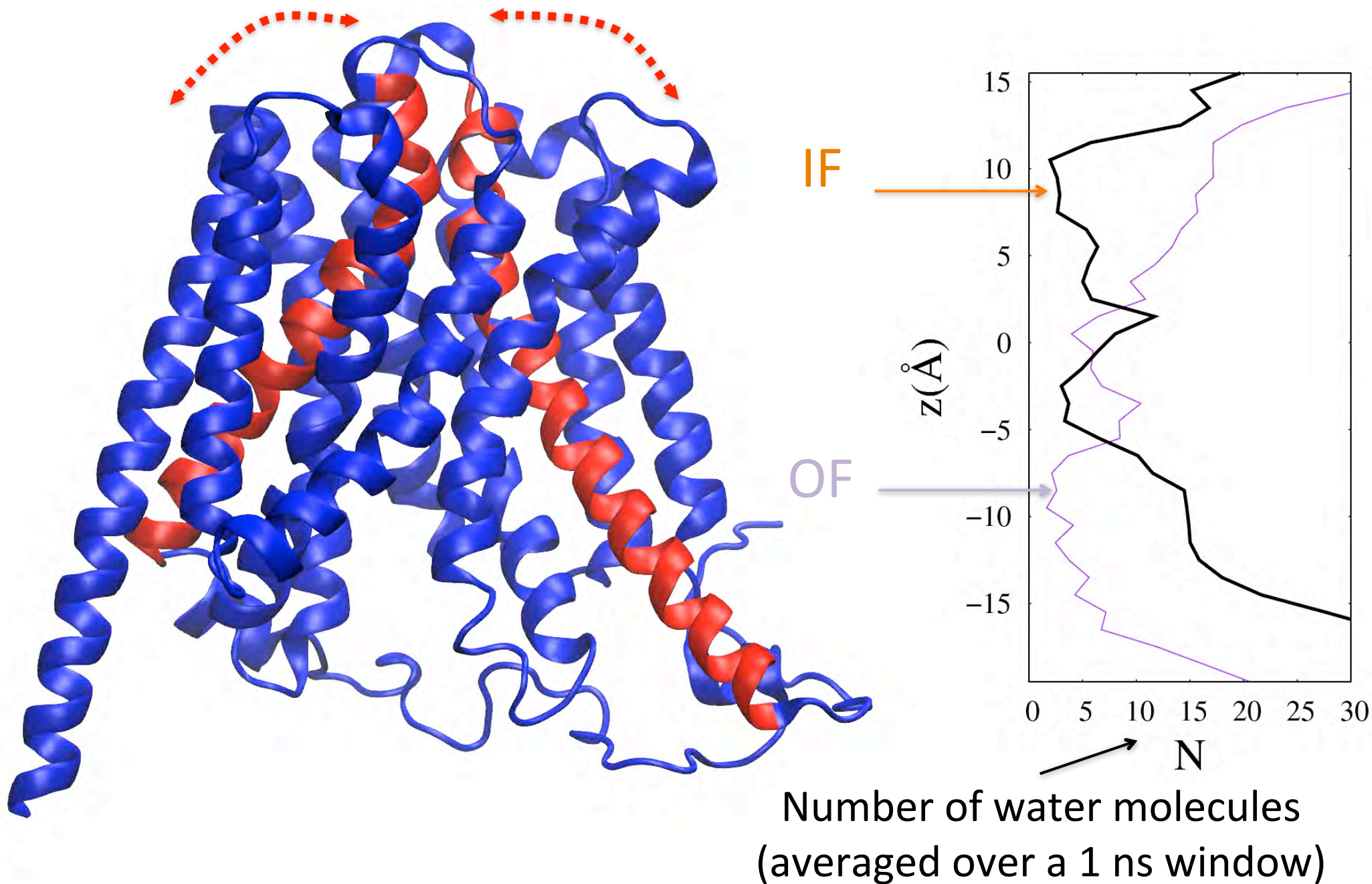
Capturing Biology at sub-Å Resolution



Large-Scale Transition of an ABC Transporter in the Membrane

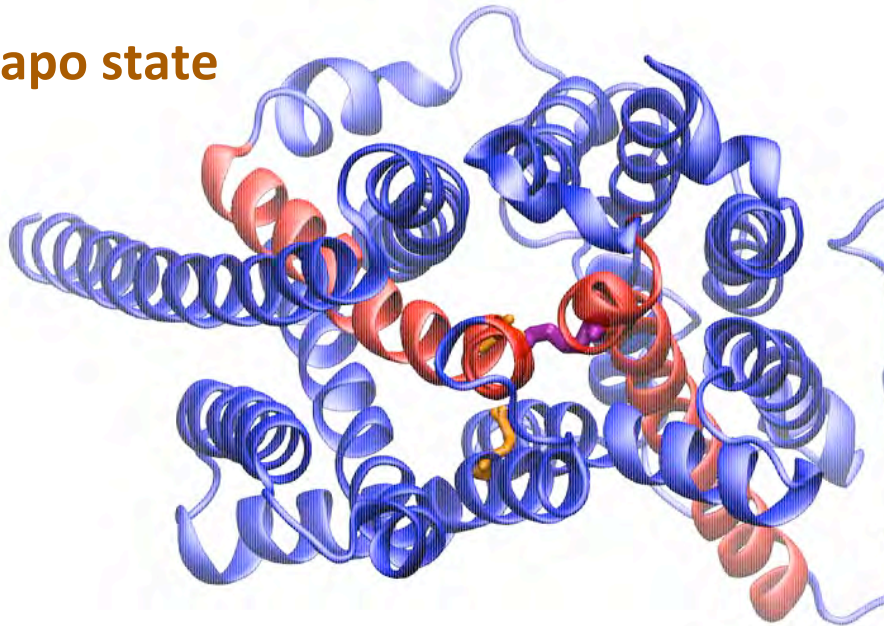


IF \leftrightarrow OF transition in an MFS Transporter in Membrane

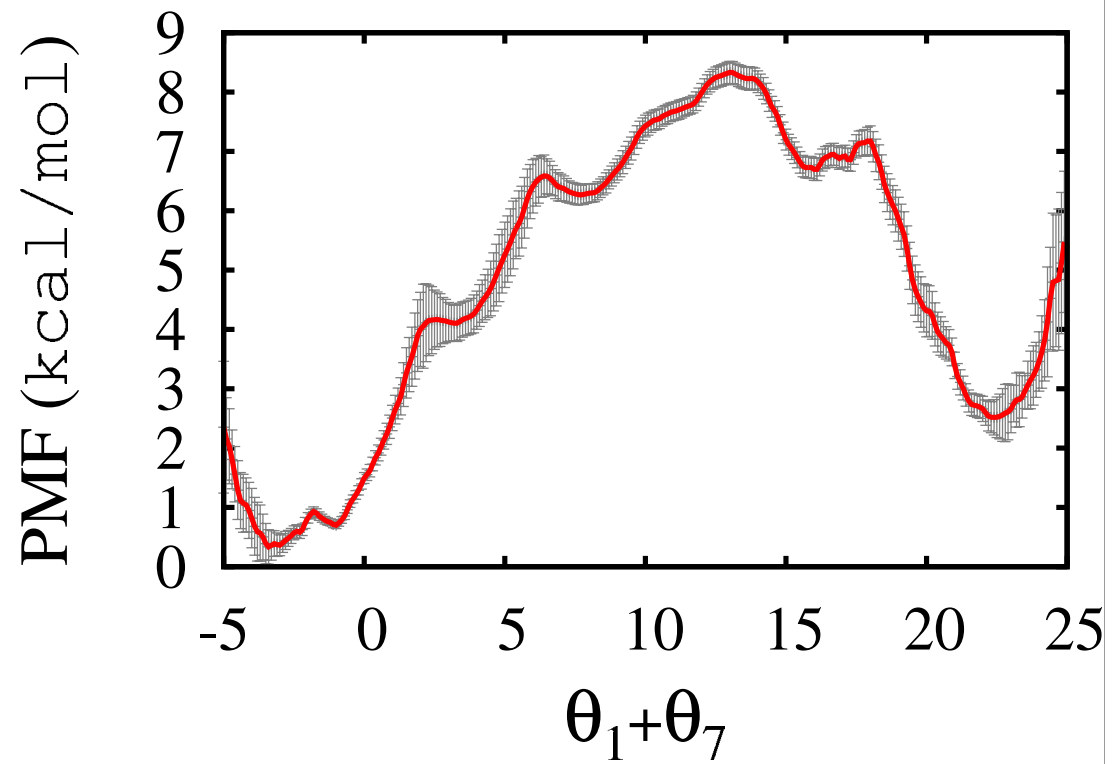
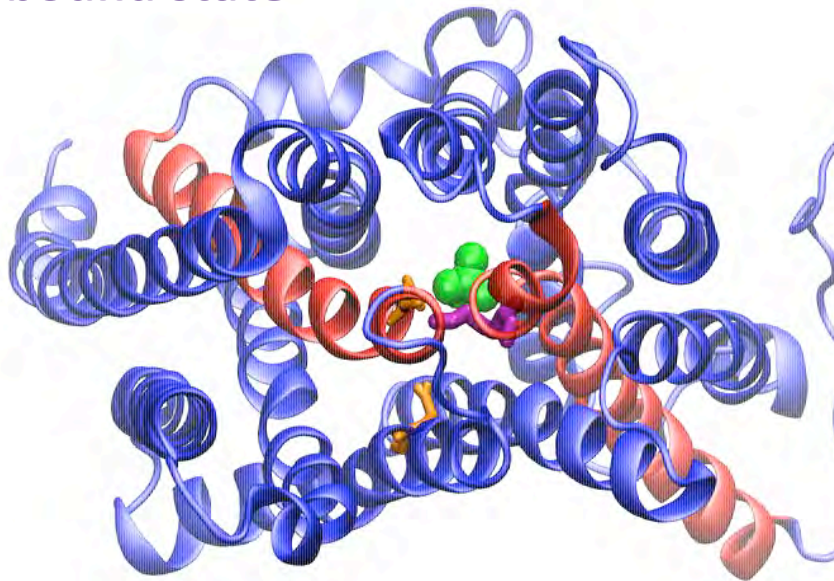


Chemomechanical Coupling in GlpT

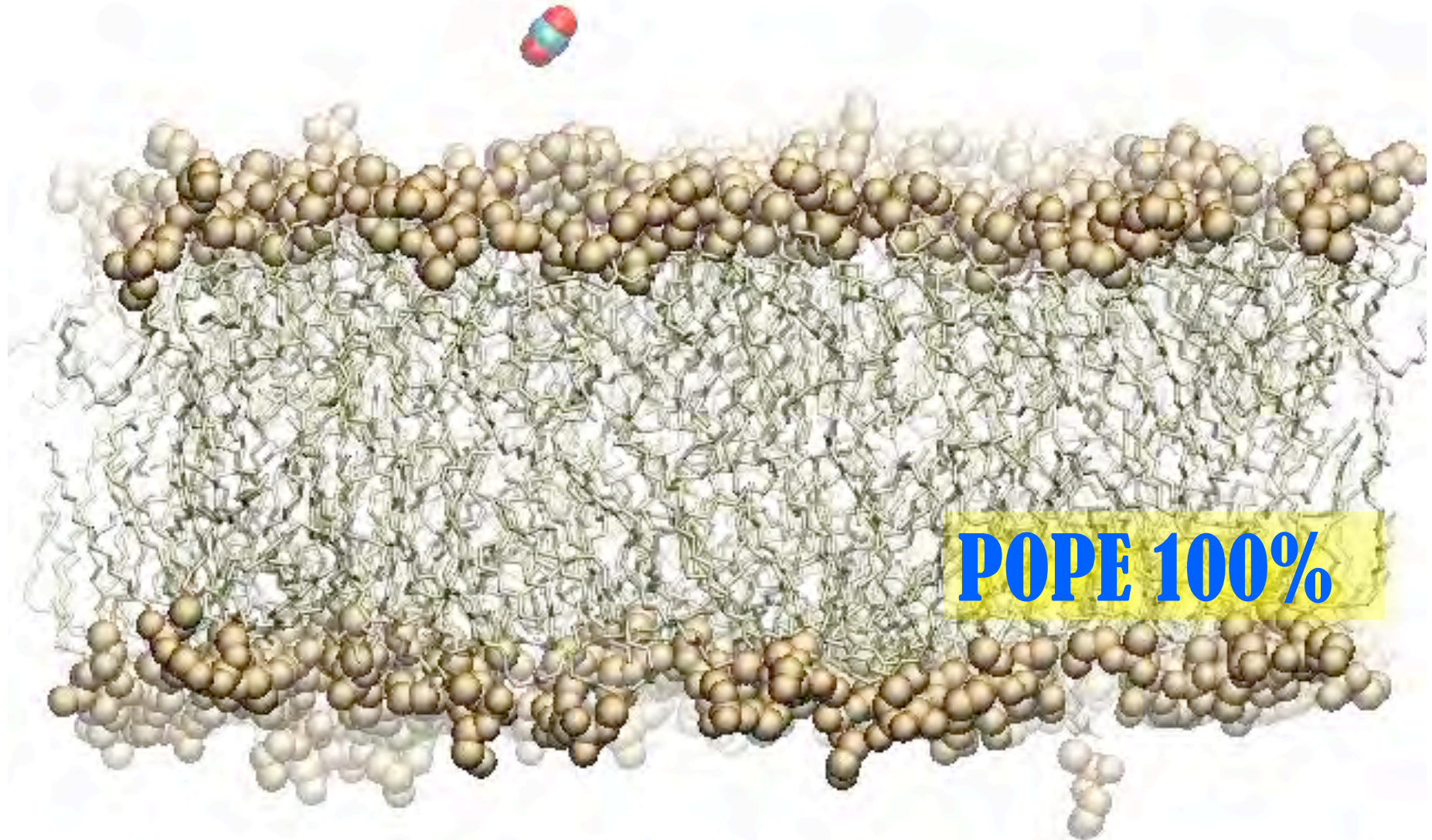
apo state



P_i-bound state

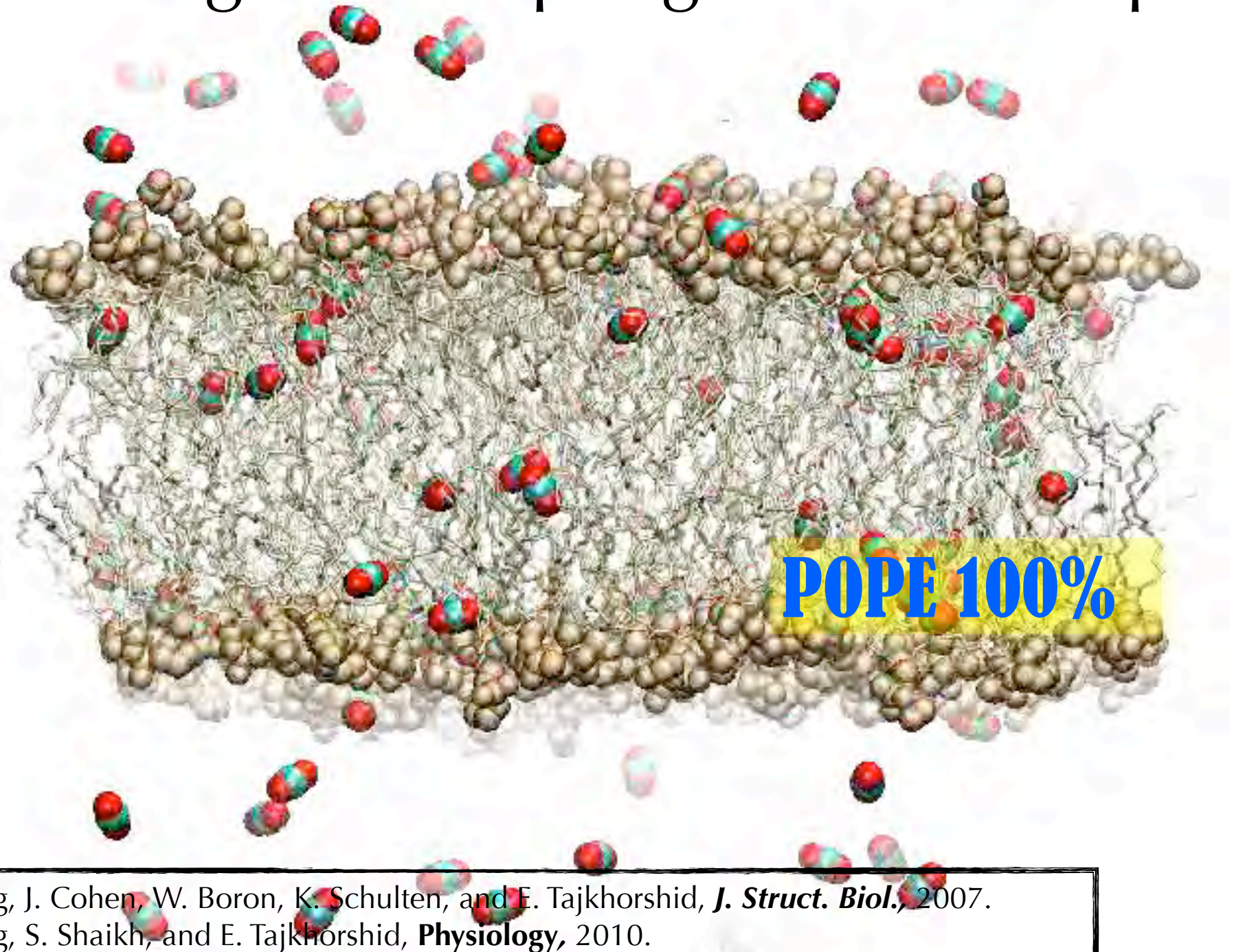


Explicit Ligand Sampling of Gas Transport



Y. Wang, J. Cohen, W. Boron, K. Schulten, and E. Tajkhorshid, *J. Struct. Biol.*, 2007.
Y. Wang, S. Shaikh, and E. Tajkhorshid, *Physiology*, 2010.

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Y. Wang, S. Shaikh, and E. Tajkhorshid, **Physiology**, 2010.

Lipid/Water Partition Coefficients

Simulation

CO ₂ in POPE	3.50
CO ₂ in POPC	2.74
O _{2(P)} in POPC	4.04
O _{2(N)} in POPC	3.46
O _{2(P)} in POPE	4.73
O _{2(N)} in POPE	5.79

Experiment

CO₂

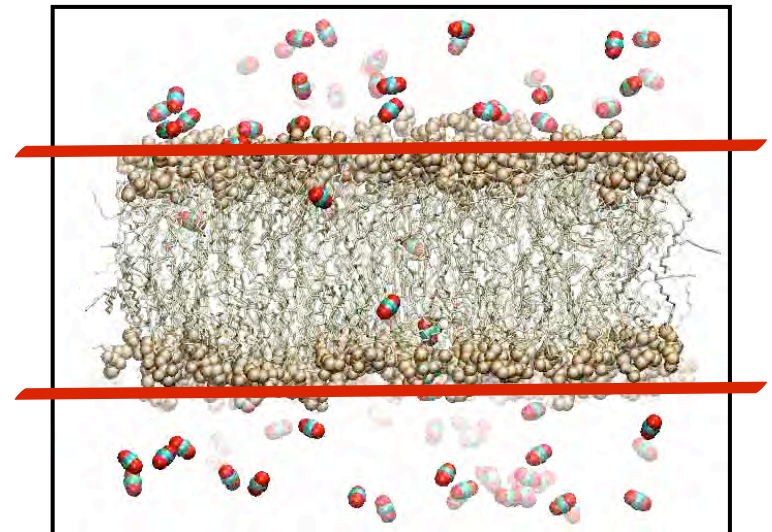
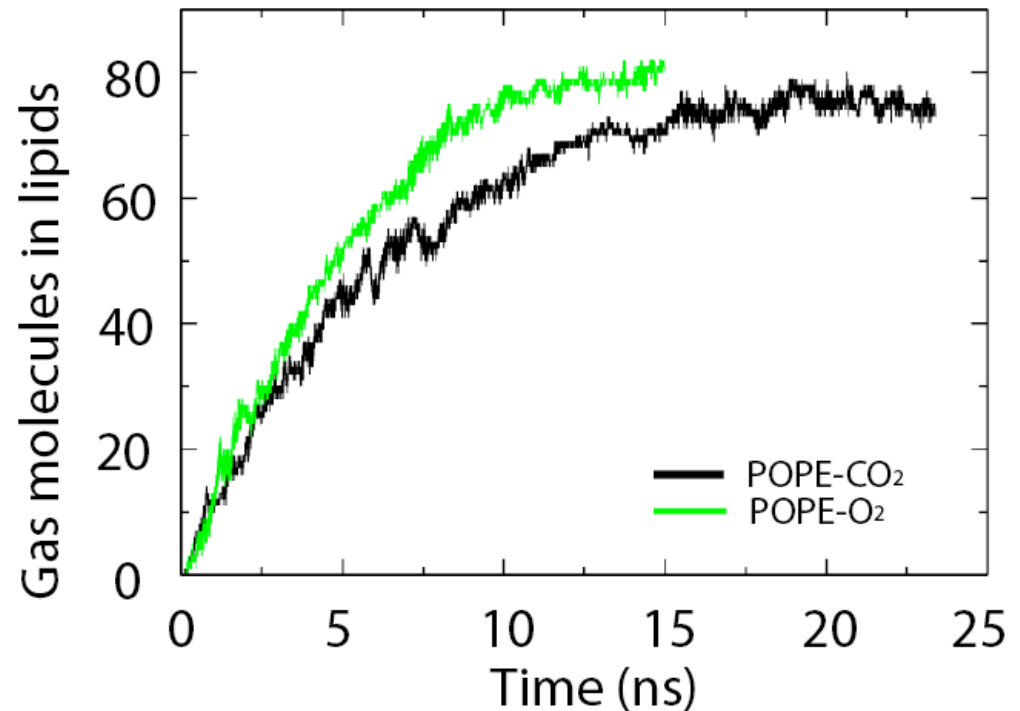
Octanol: 1.3

Hexadecane: 1.5

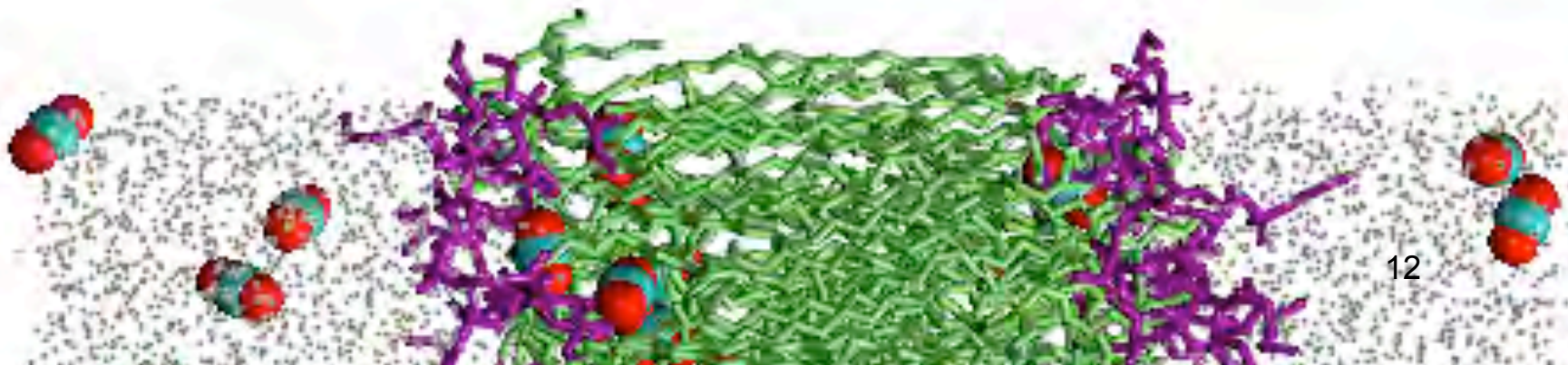
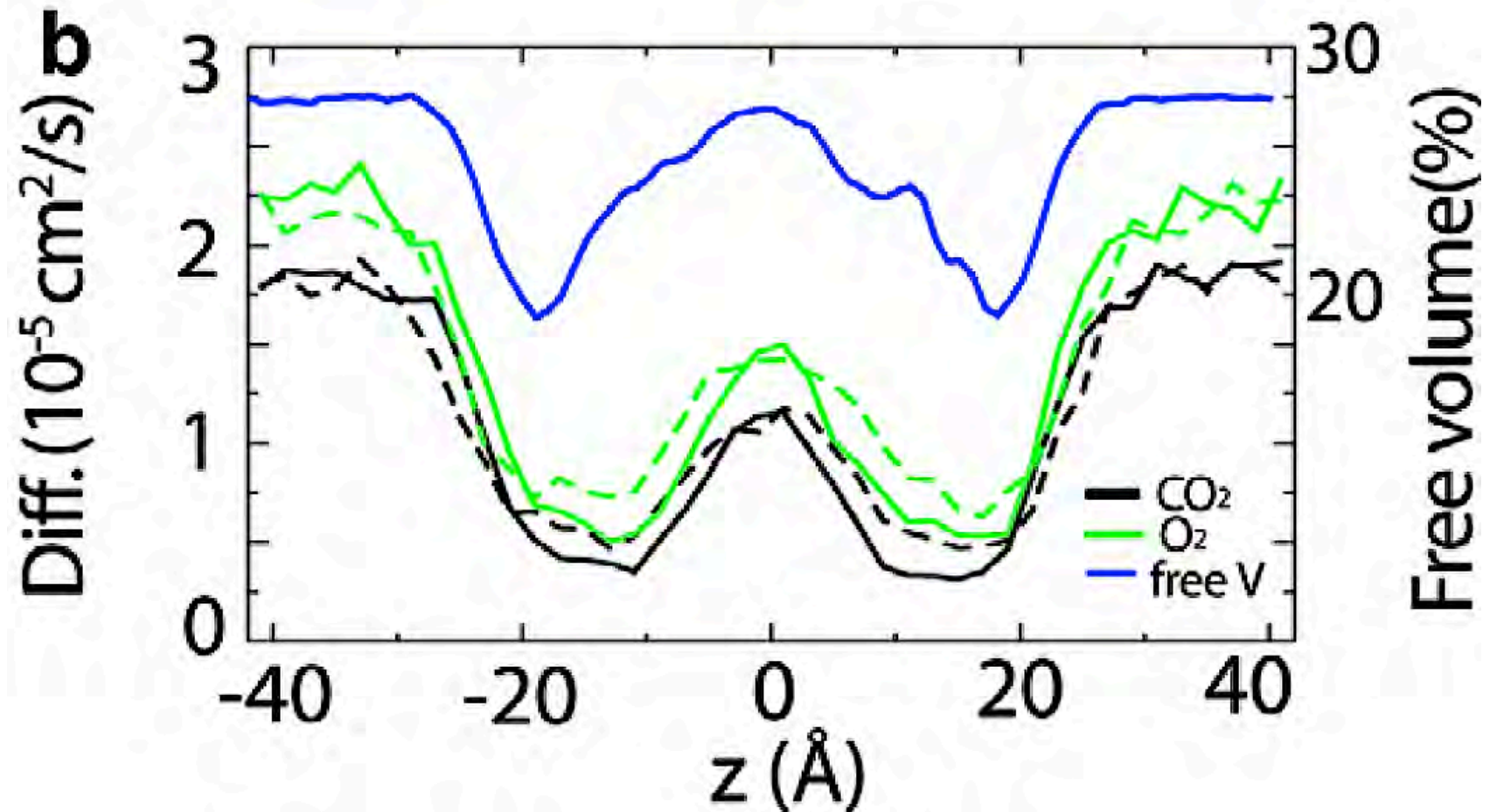
Olive oil: 1.7

O₂

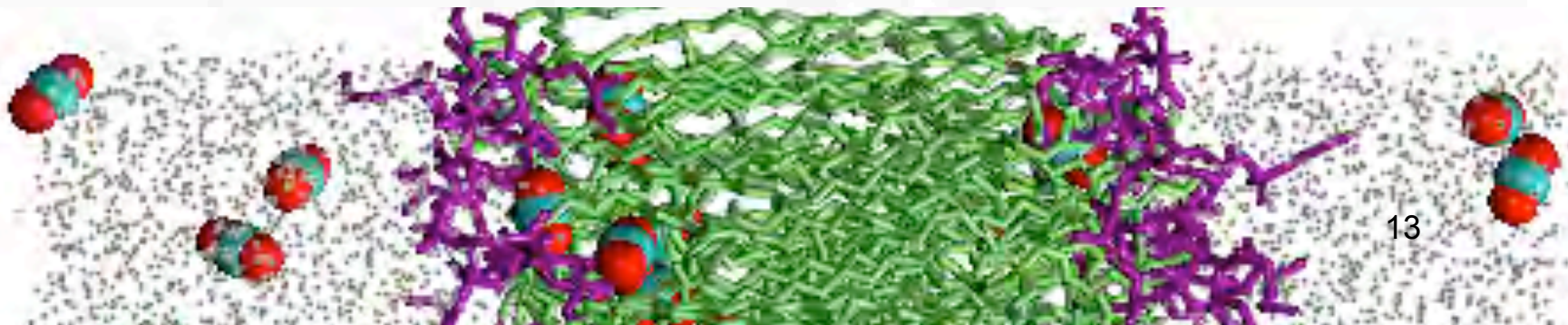
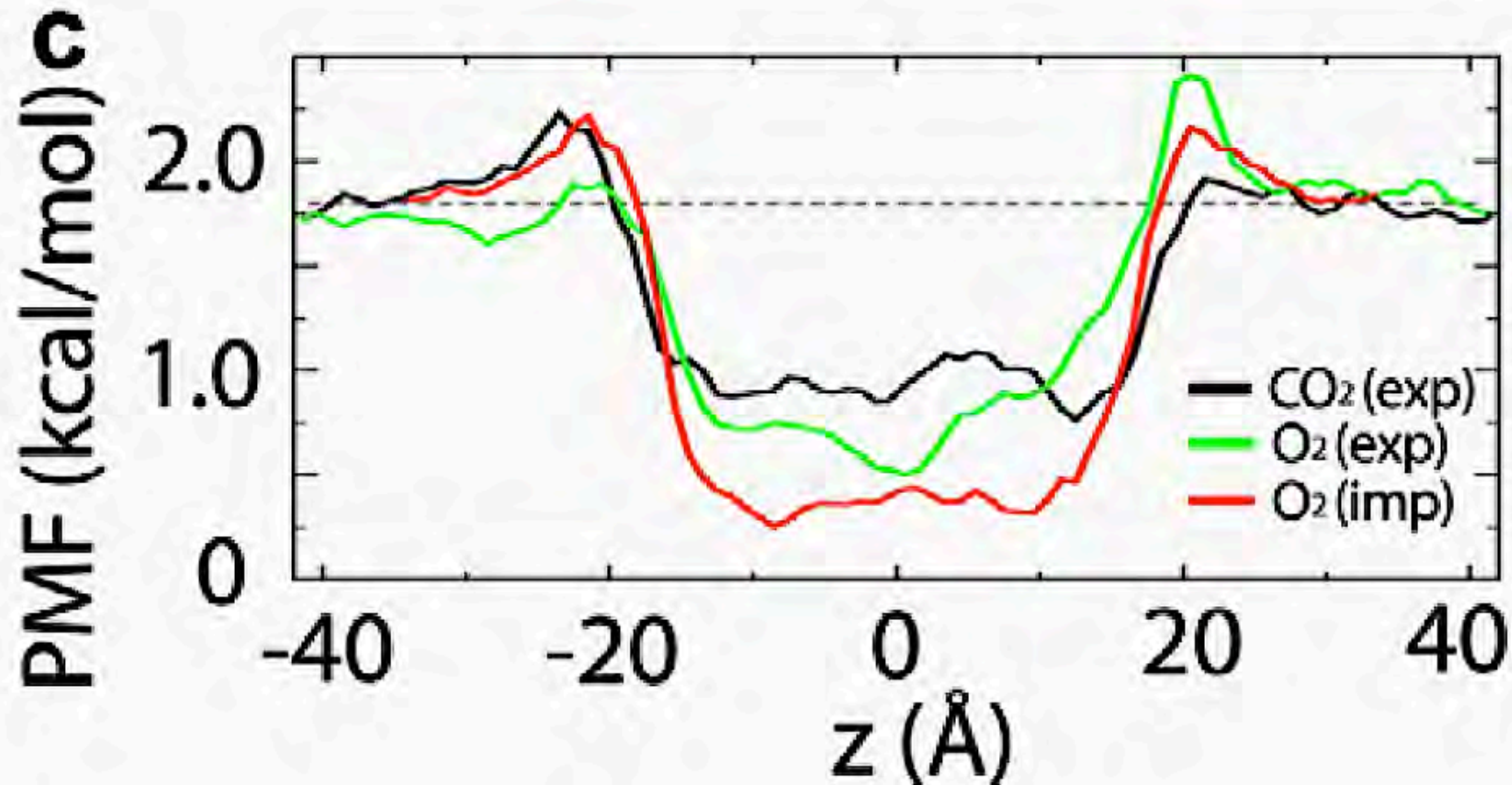
Liposome: 3.9



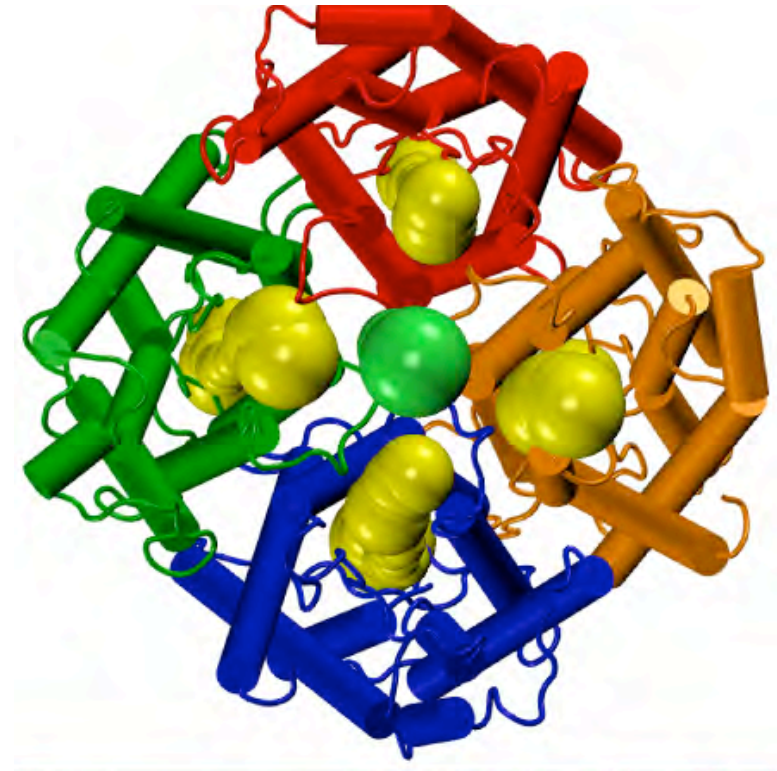
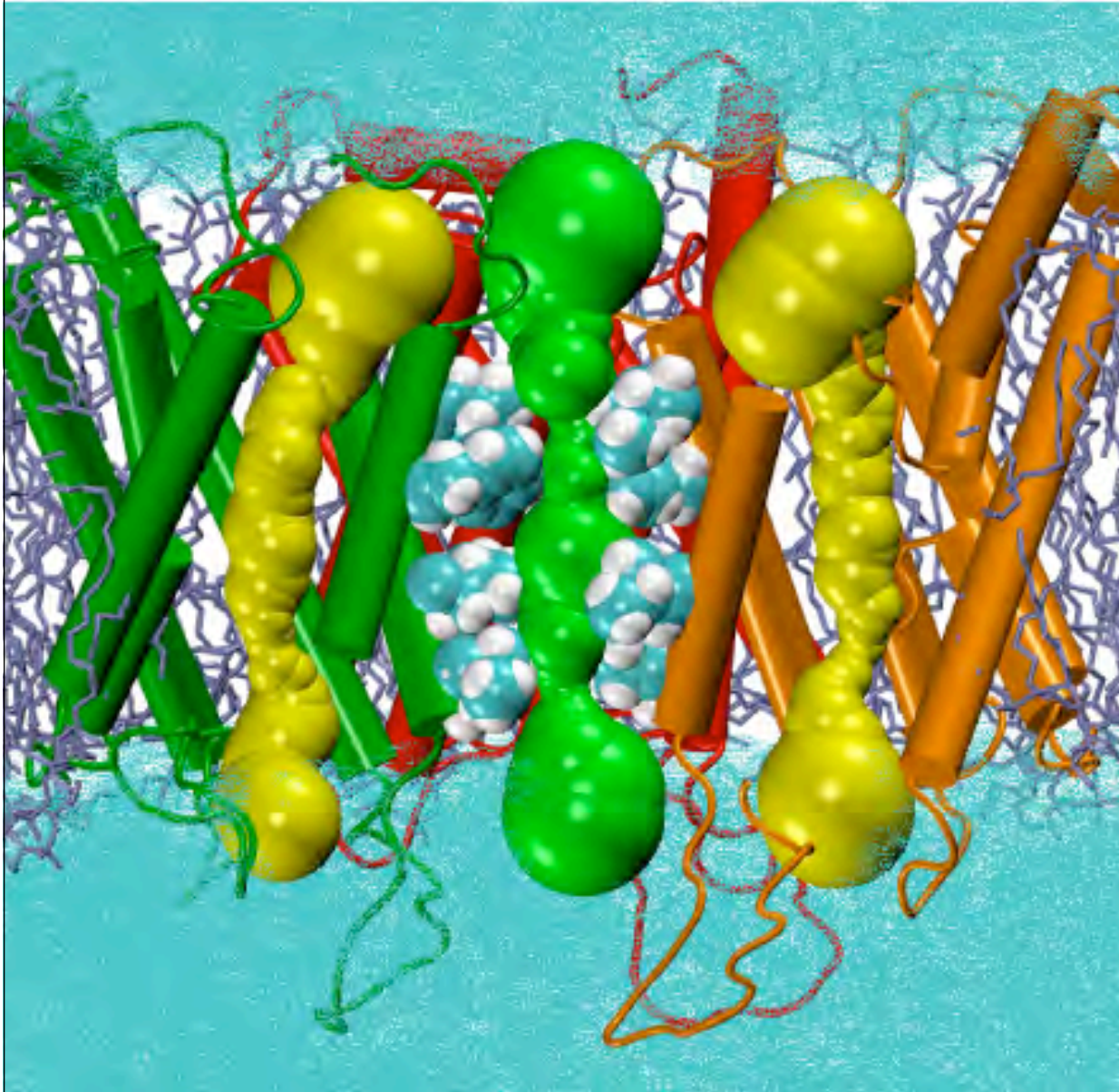
Gas Diffusion Inside the Lipid Bilayer



Gas Diffusion Inside the Lipid Bilayer

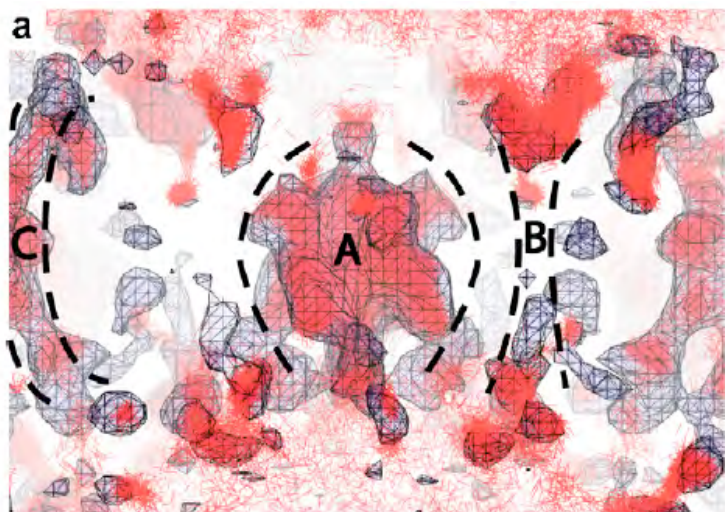


Aquaporin **Water**/**Gas** Channels

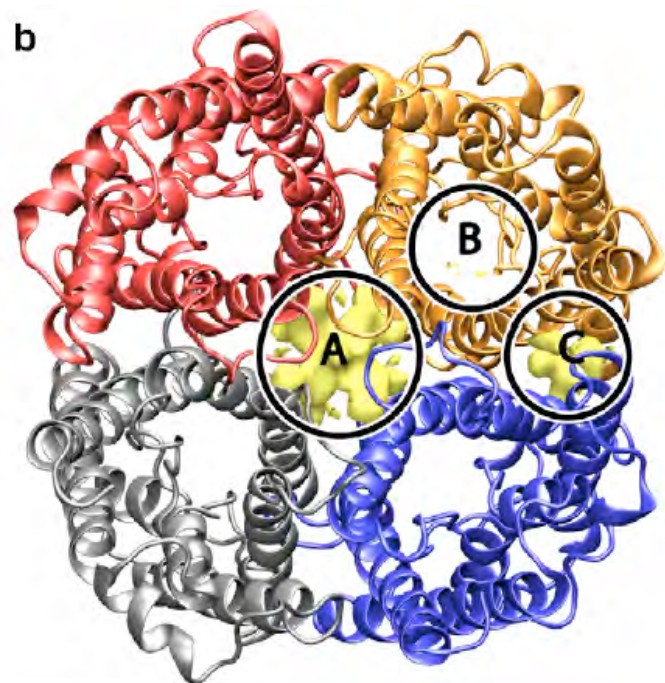


**Why
Tetramers?**

Implicit Ligand Sampling



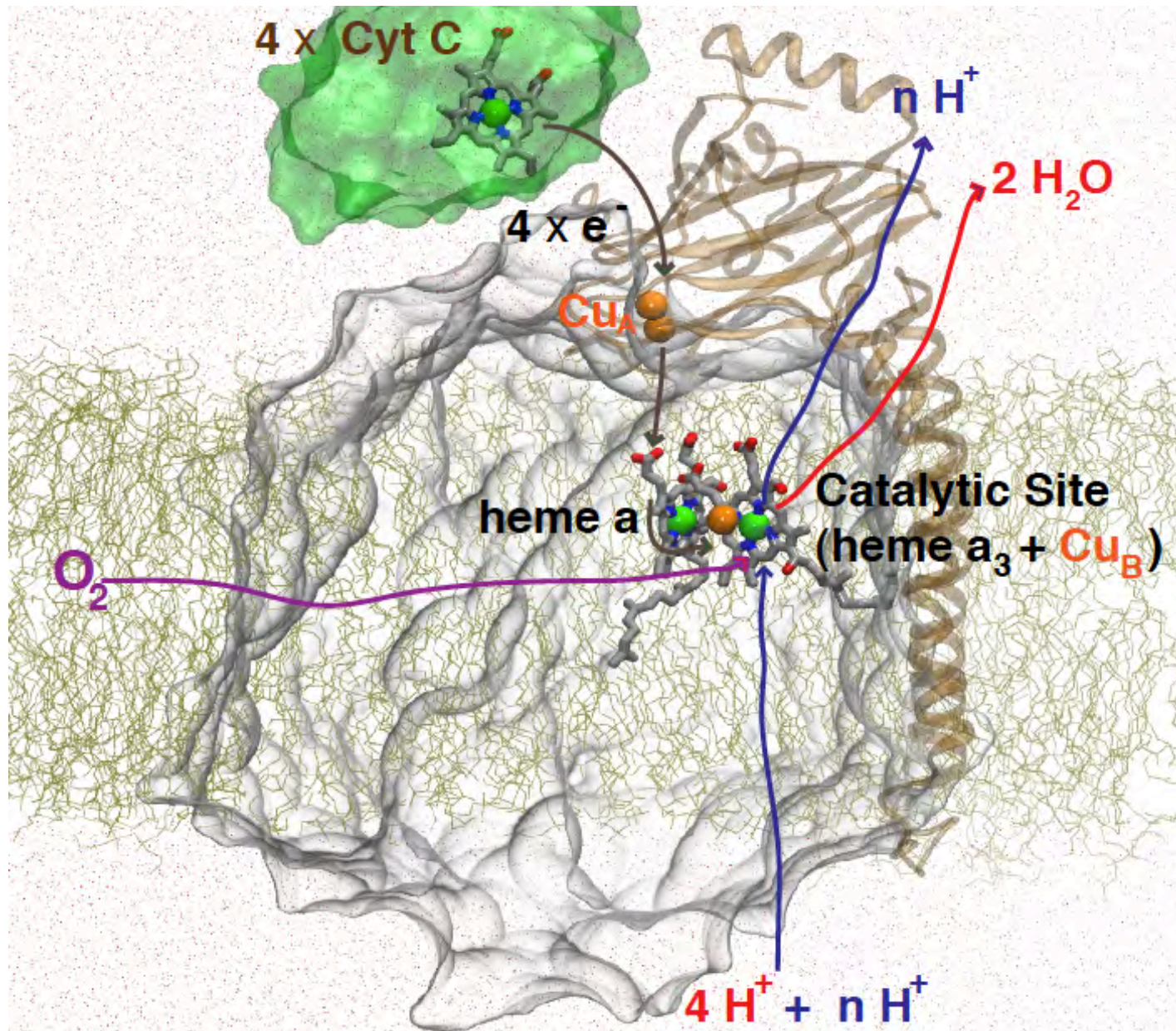
$$\mathcal{W}(\mathbf{r}) = -k_B T \ln \left[\frac{\rho(\mathbf{r})}{\rho_o} \right]$$



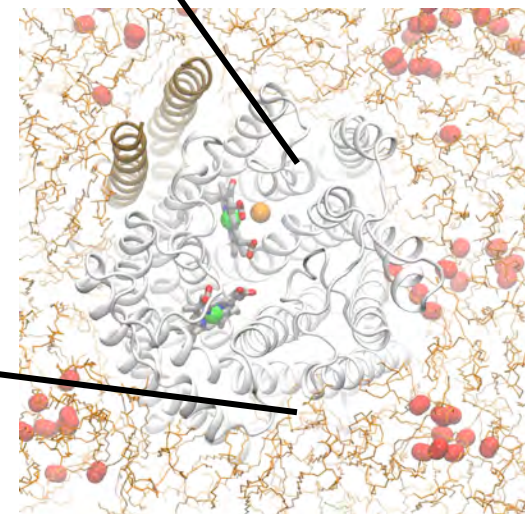
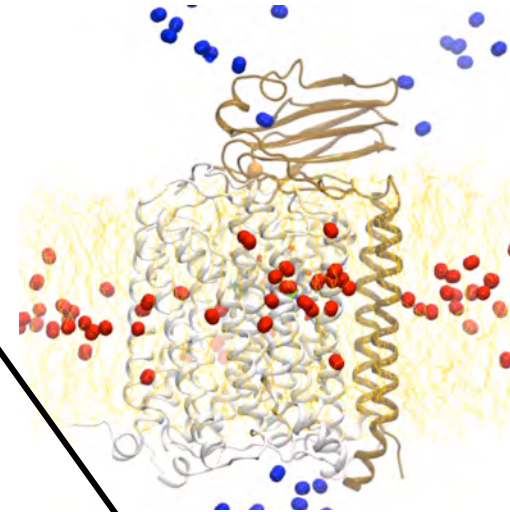
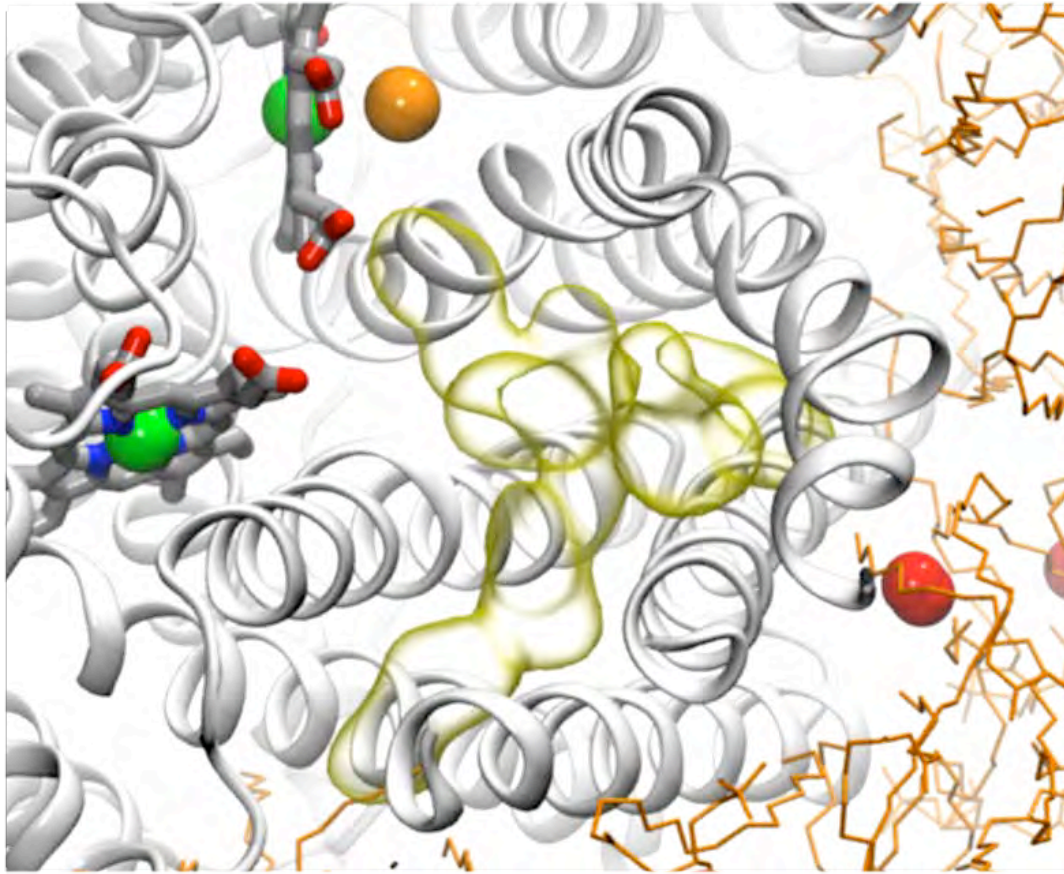
$$F(z) = -RT \ln \sum_{x,y=0}^{L_x,L_y} \frac{e^{-F(x,y,z)/RT}}{L_x L_y}$$

Cohen, et al., 2006; Wang, et al., 2007

Oxido-reductase and Proton Pump

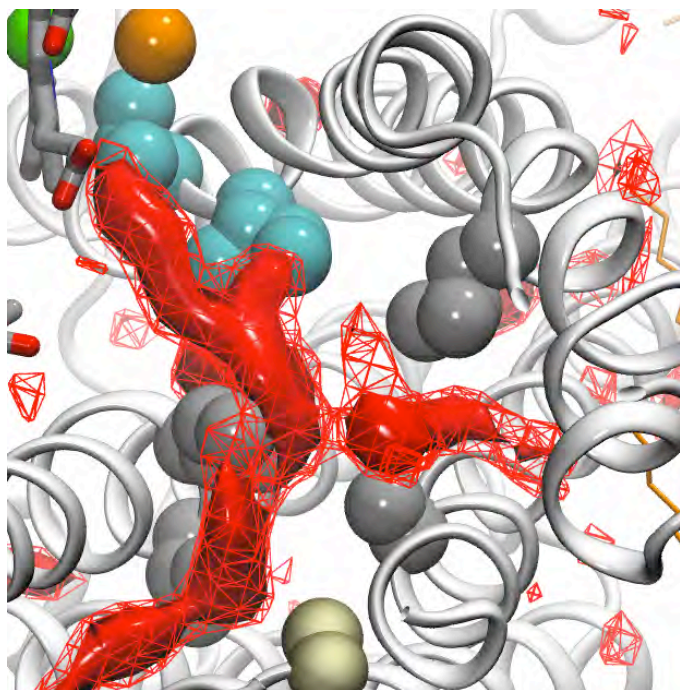


Rapid O₂ Permeation via the Hydrophobic Channel in Cytochrome C Oxidase

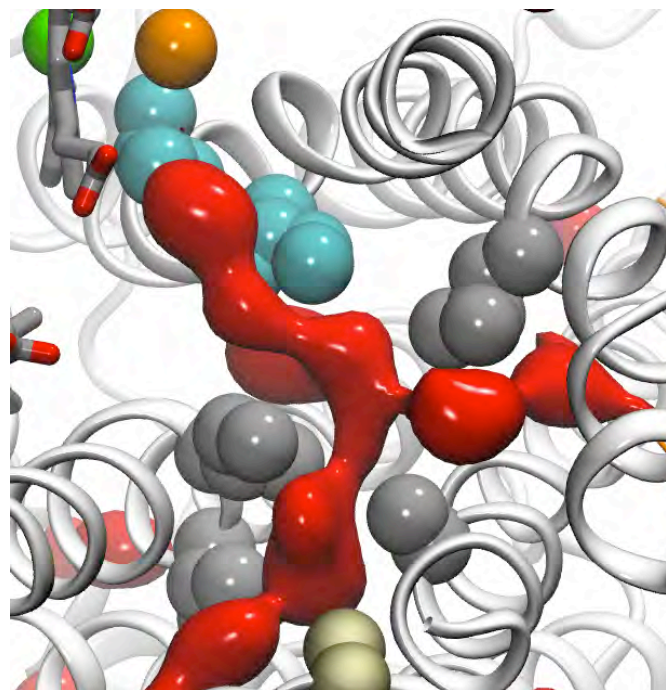


O₂ Pathway in Cytochrome C Oxidase

Implicit ligand sampling

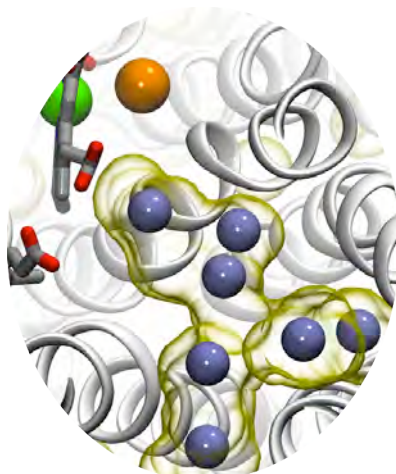


Explicit O₂ simulation



Reddish solid: $\Delta\Delta G$ map of ~ -3.5 kcal/mol ; Reddish wireframe: $\Delta\Delta G$ map of ~ -3.0 kcal/mol

Observed Xenon
binding in CcO ba₃
crystal structures

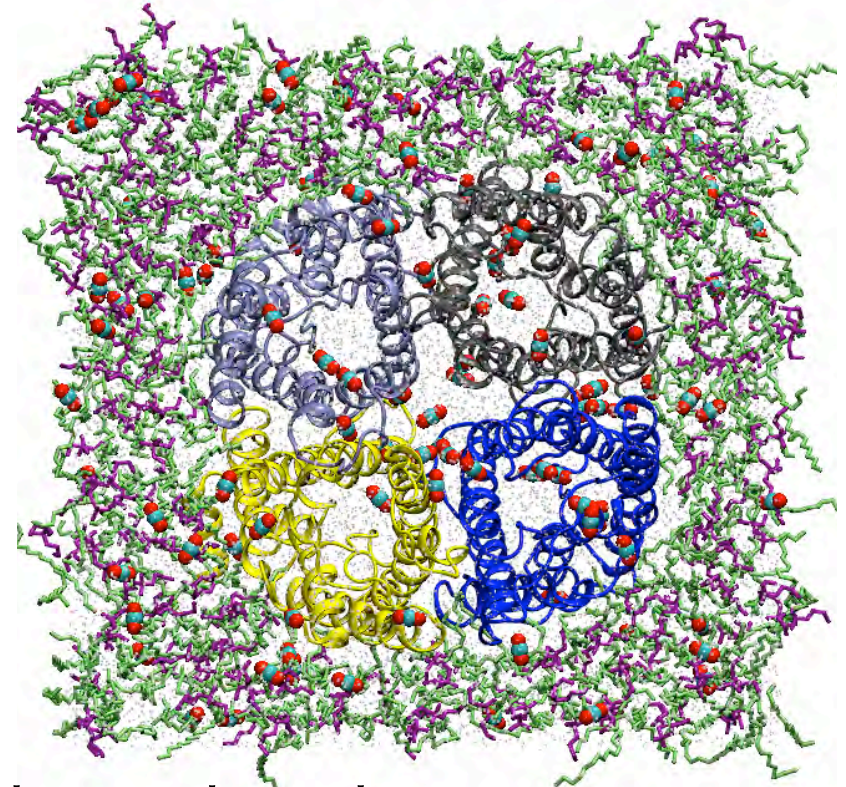
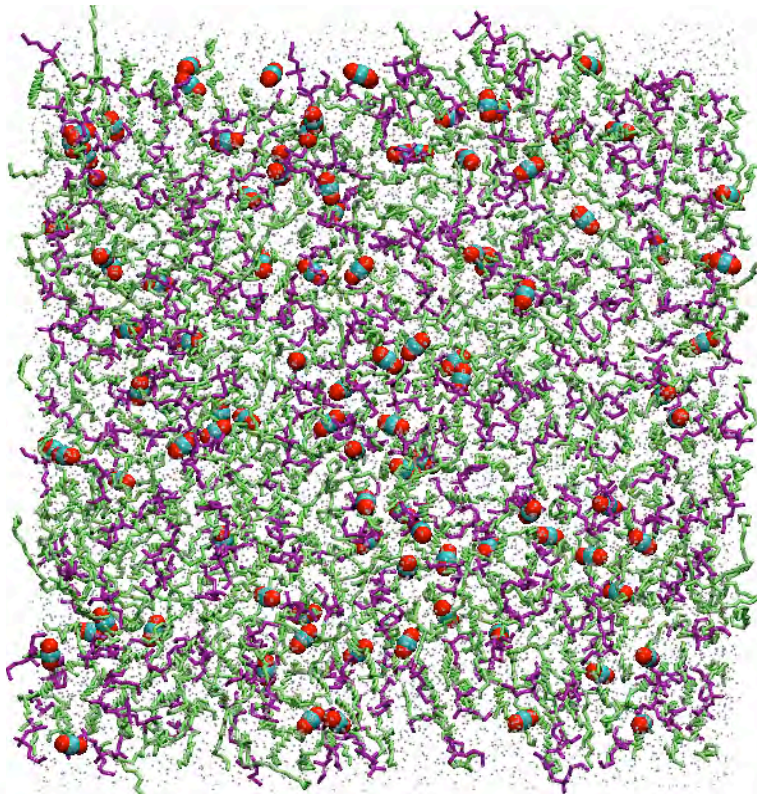


All located along the hydrophobic channel

Luna VM, Chen Y., Fee JA and Stout CD (2008)
Biochemistry, 47, 4657-4665 (PDB entry 3BVD)

Luna VM, Fee JA, Deniz AA and Stout CD (2012)
Biochemistry, 51, 4669-4676

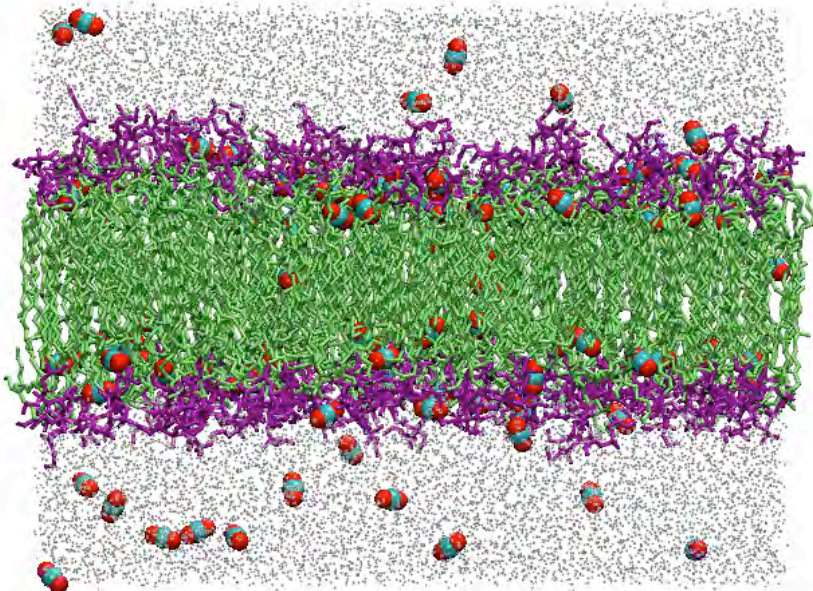
Simulating Membrane Gas Transport



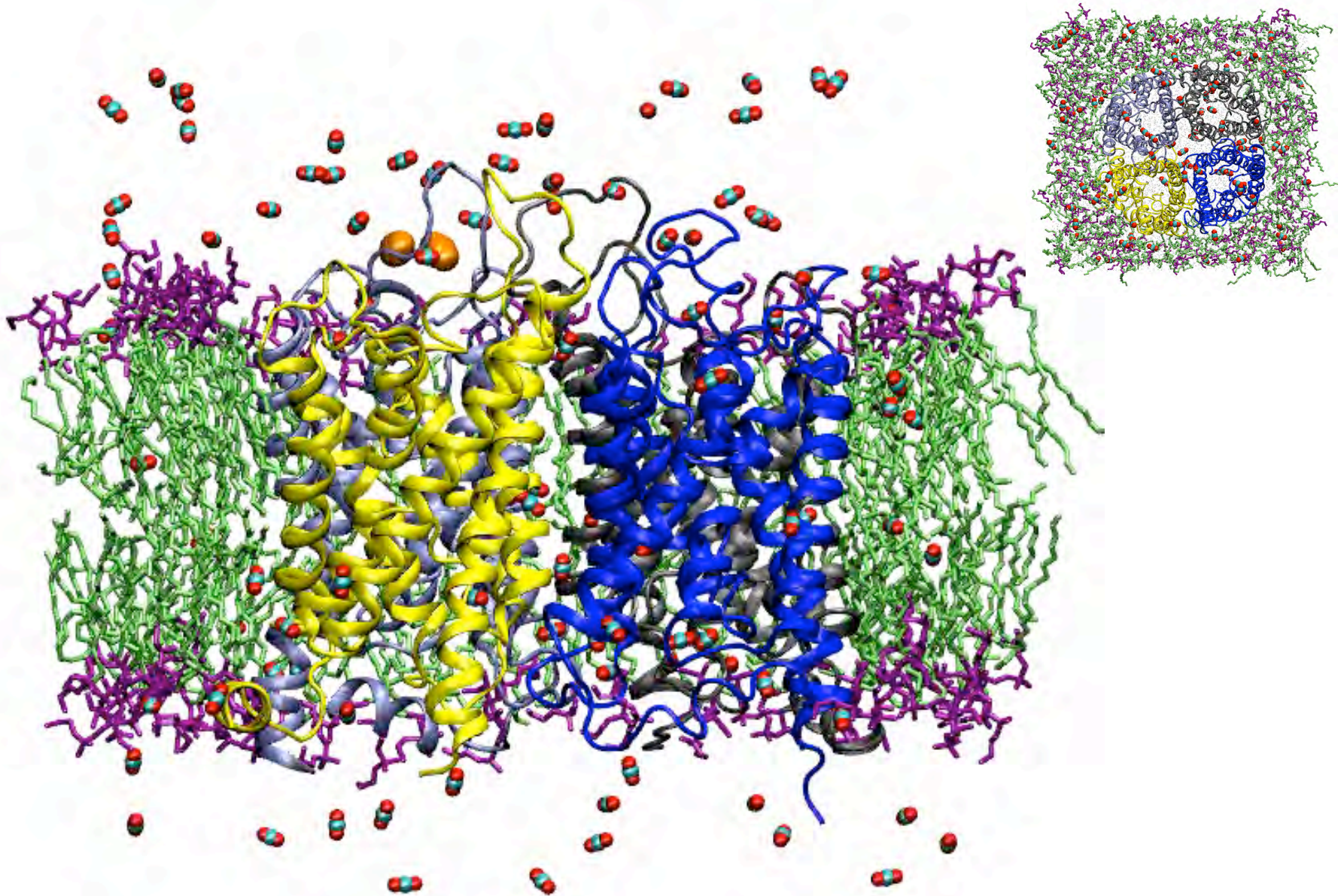
Identical total areas

Calculating permeation rate
in MD simulations

AQP1	AQP4
CO ₂	O ₂ NO

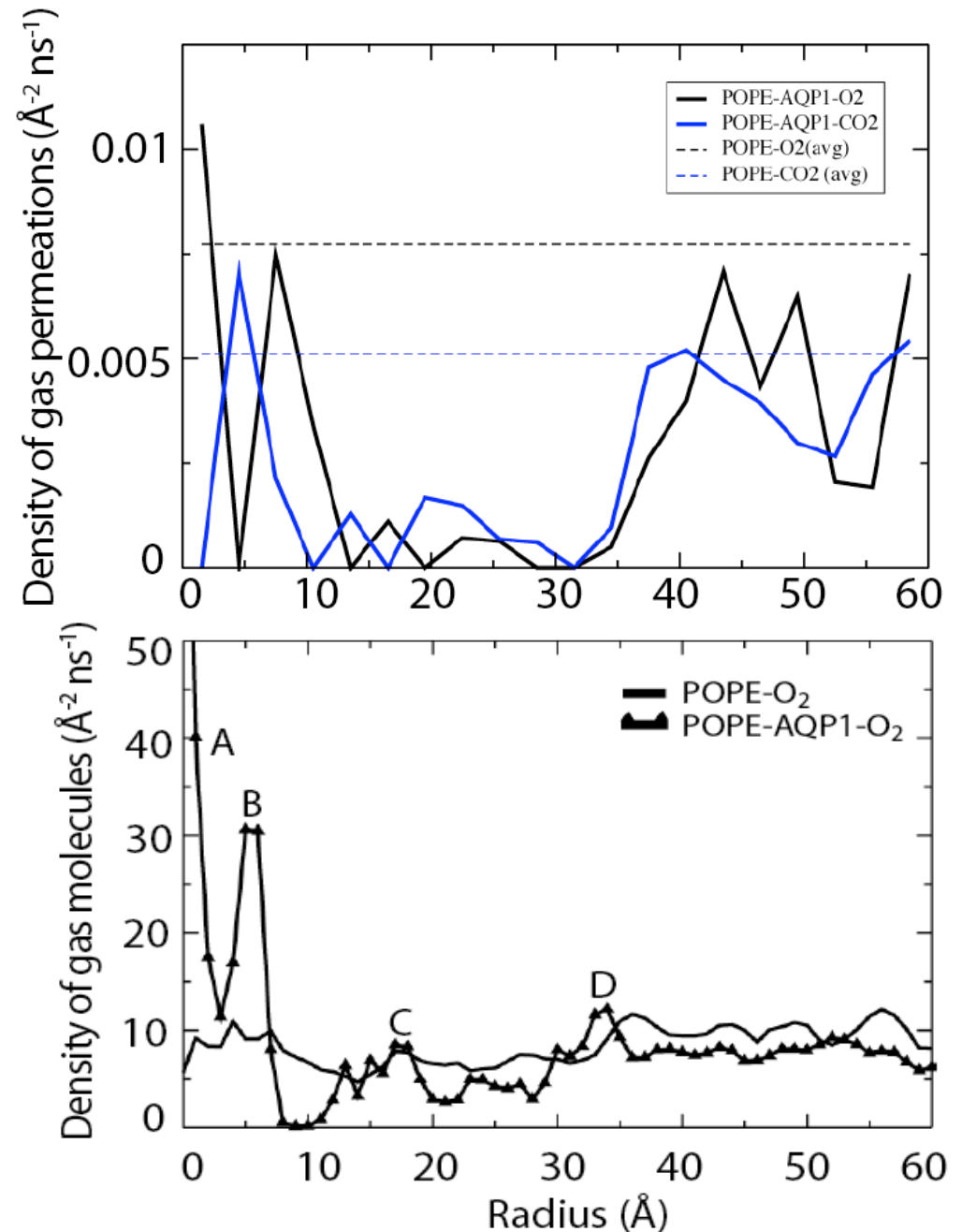
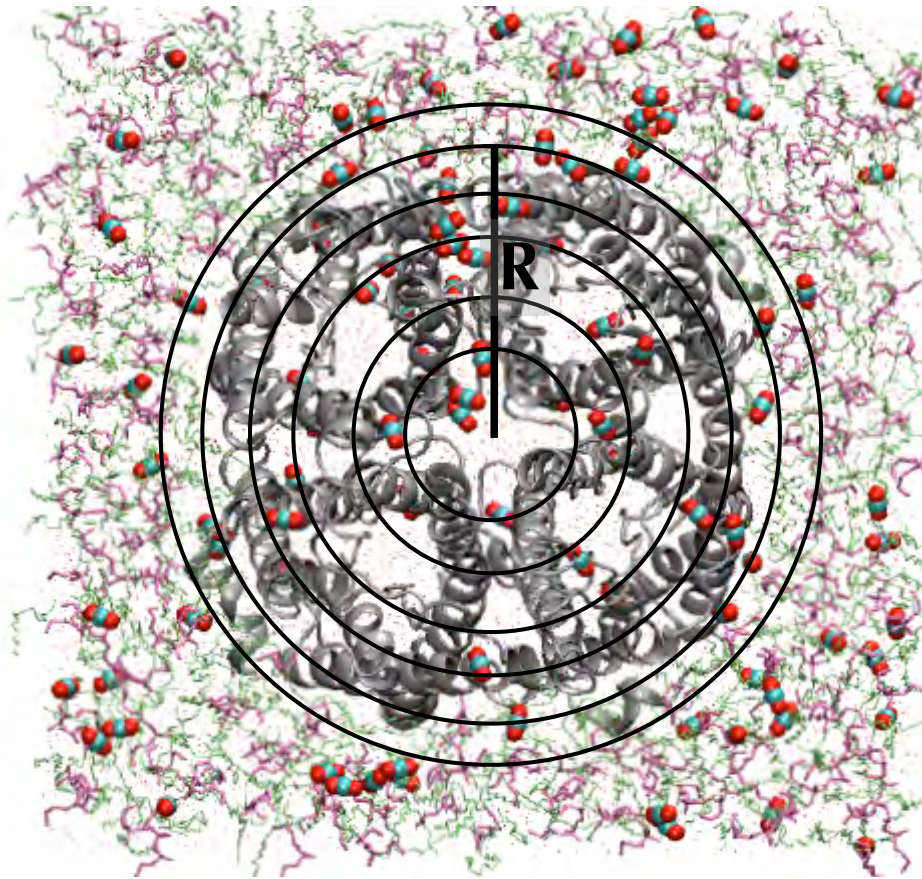


Gas Transport through Aquaporins

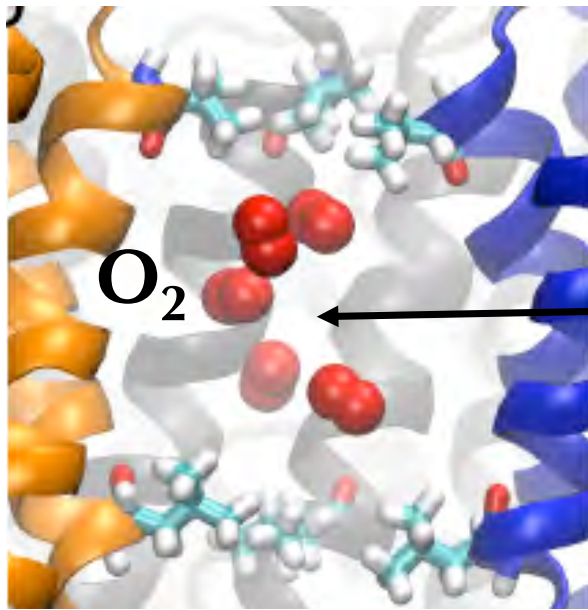


Typical permeation events (300-400 ps)

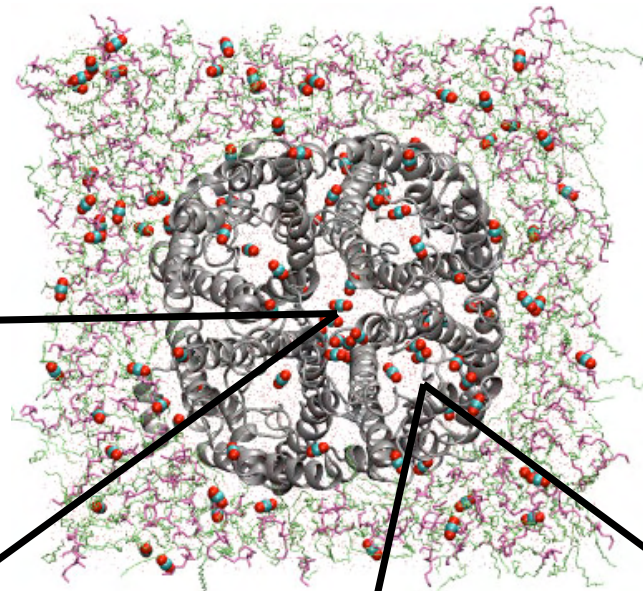
Gas Occupancy/Permeation Radial Distribution



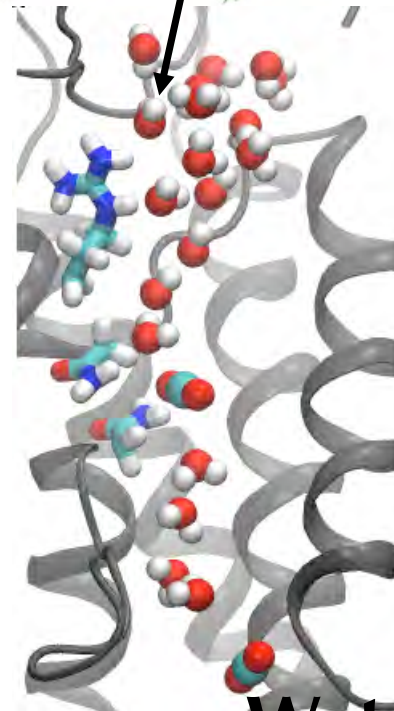
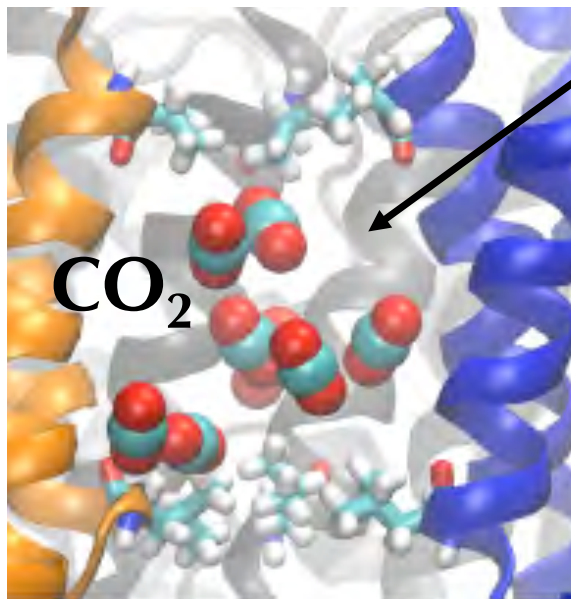
A Role for the Central Pore!



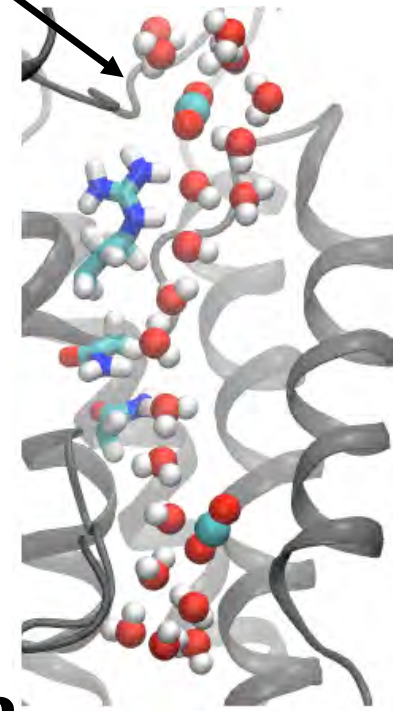
Central Pore



CO_2

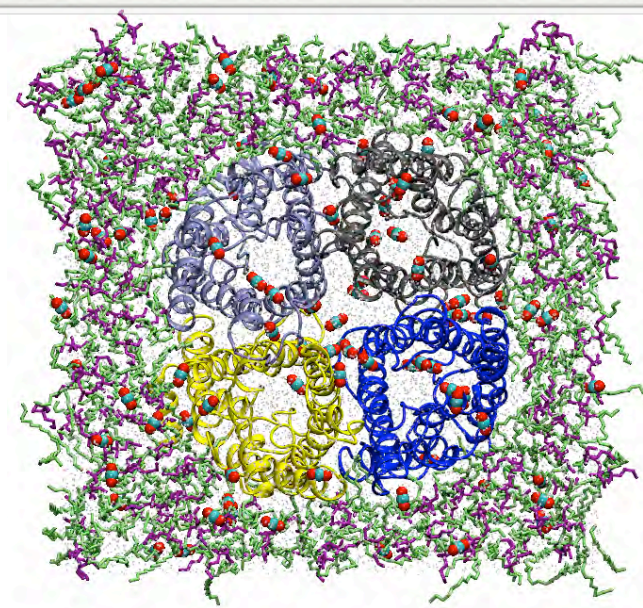
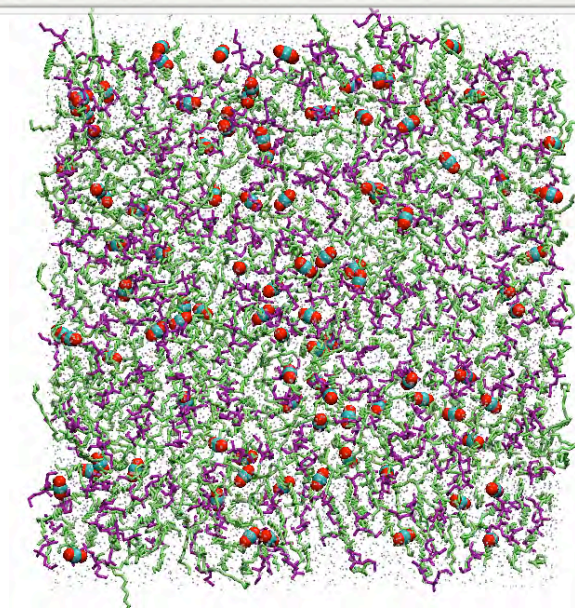


Water Pores

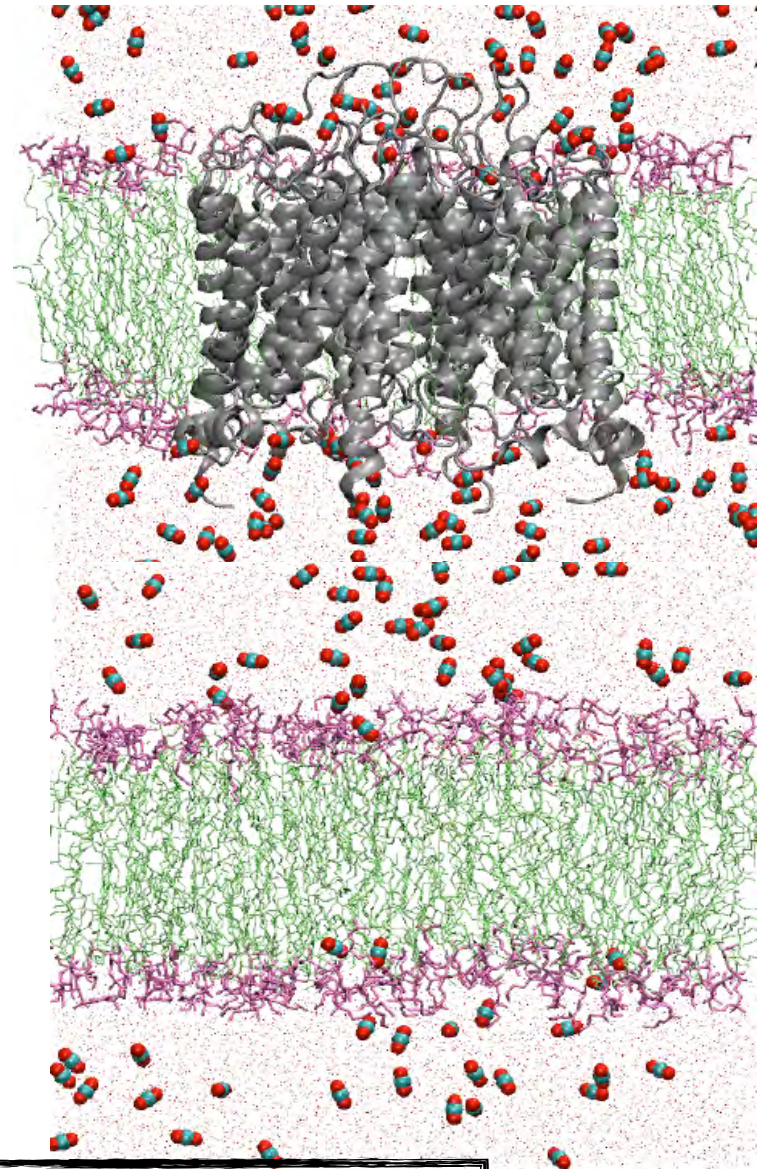
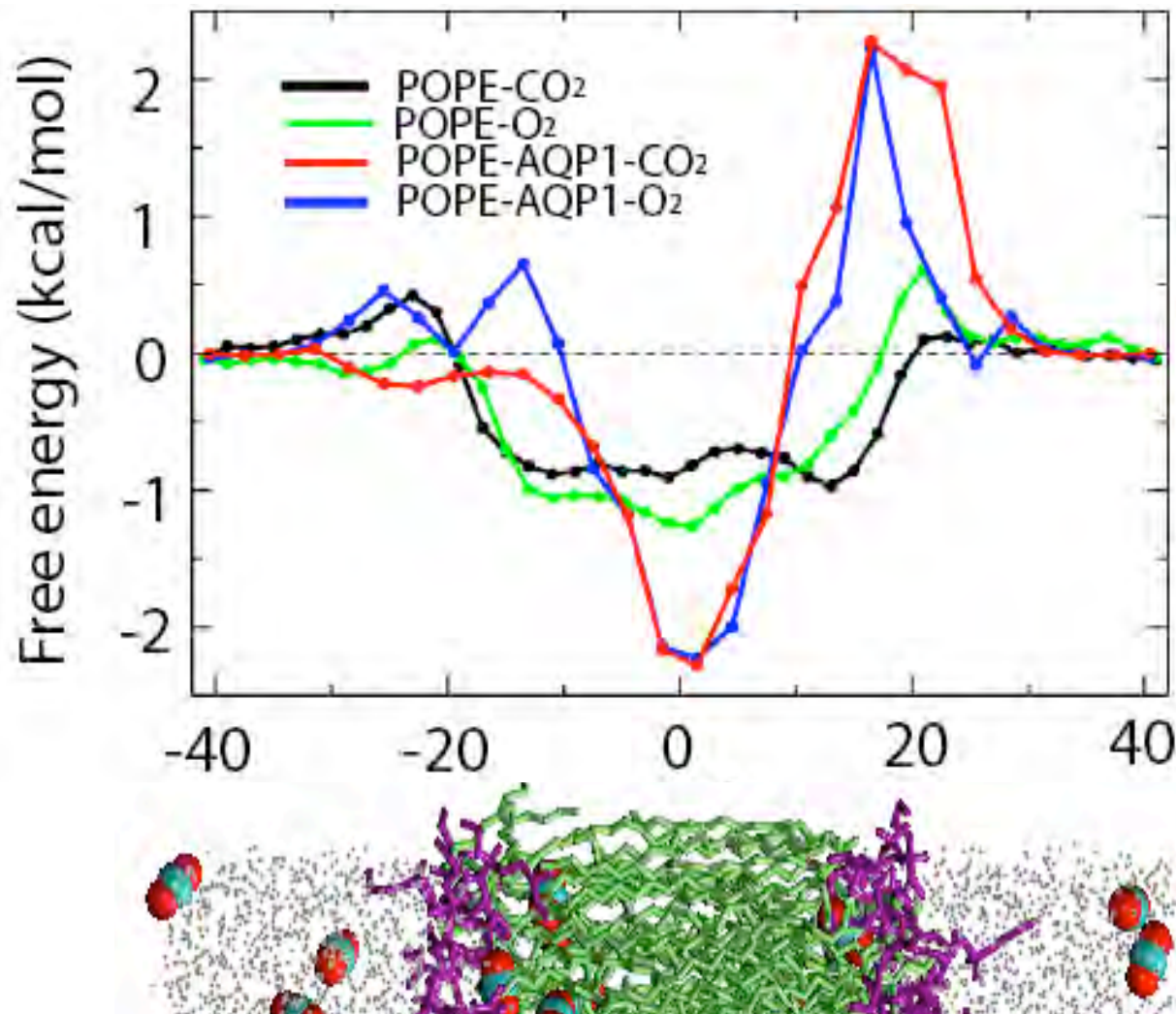


Gas Transport through Aquaporins

SYSTEM	TOTAL (100x100 Å ²)	WATER PORES (4)	CENTRAL PORE (1)
<i>Equi POPE-CO₂</i>	3	N/A	N/A
<i>Equi POPC-CO₂</i>	5	N/A	N/A
<i>Equi POPC-O_{2(P)}</i>	16	N/A	N/A
<i>Equi POPE-O_{2(P)}</i>	11	N/A	N/A
Press POPE-CO ₂	168	N/A	N/A
Press POPC-CO ₂	160	N/A	N/A
Press POPE-O _{2(P)}	310	N/A	N/A
Press POPC-O _{2(P)}	208	N/A	N/A
Press POPE-AQP1-CO₂	76	6	4
Press POPE-AQP1-O_{2(P)}	79	1	6



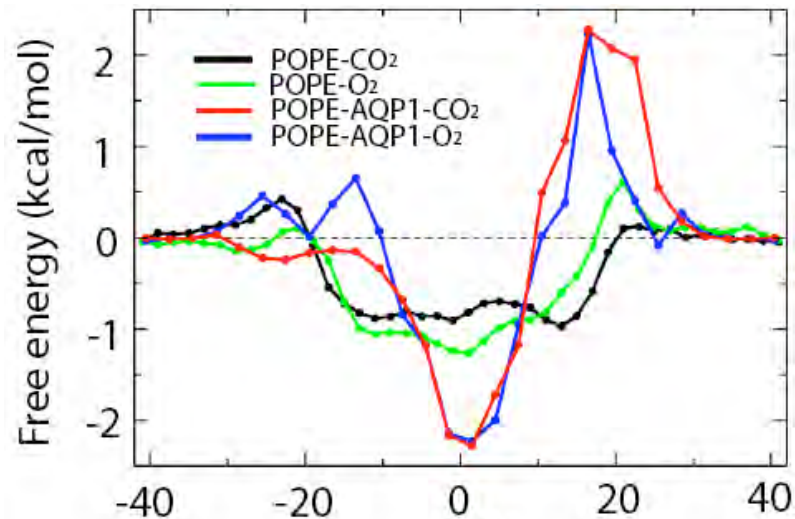
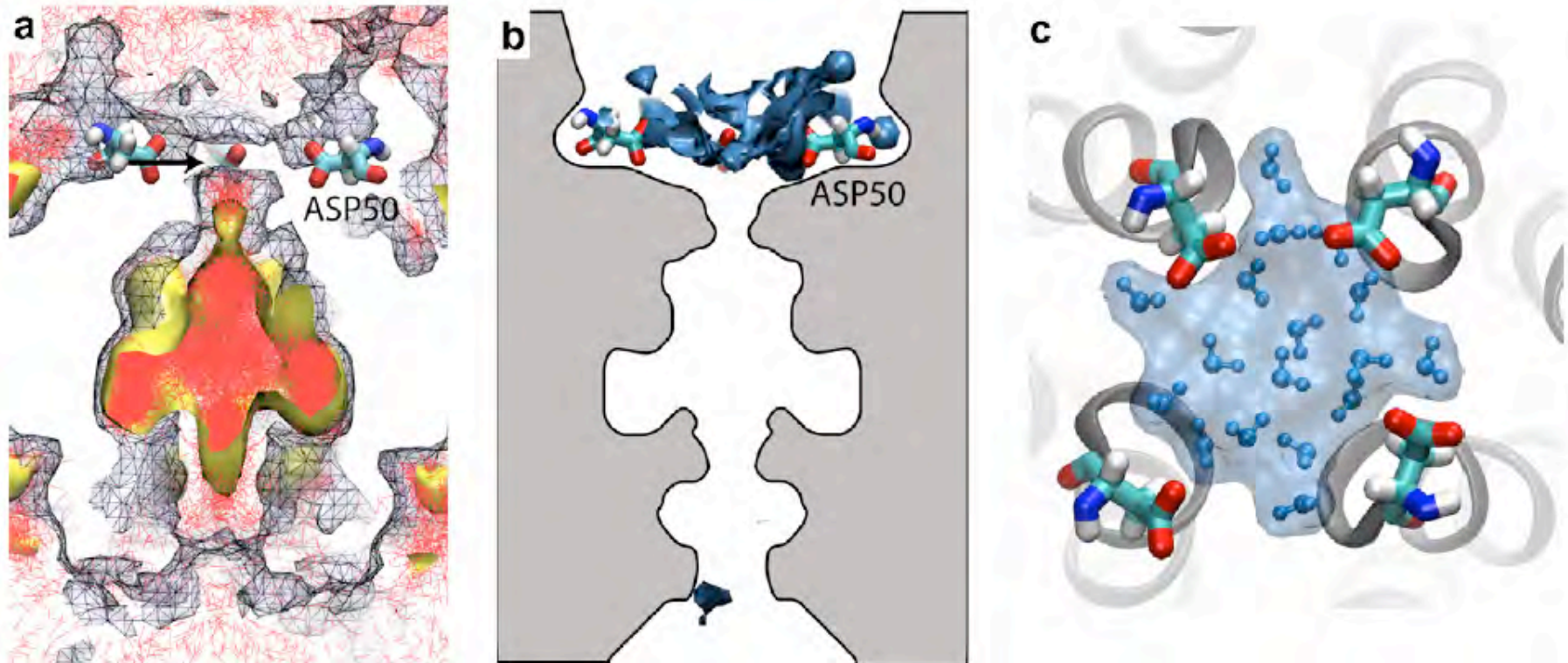
Free Energy Profiles for O₂ and CO₂



Y. Wang, J. Cohen, W. Boron, K. Schulten, and E. Tajkhorshid, *J. Struct. Biol.*, 2007.

Y. Wang, S. Shaikh, and E. Tajkhorshid, *Physiology*, 2010.

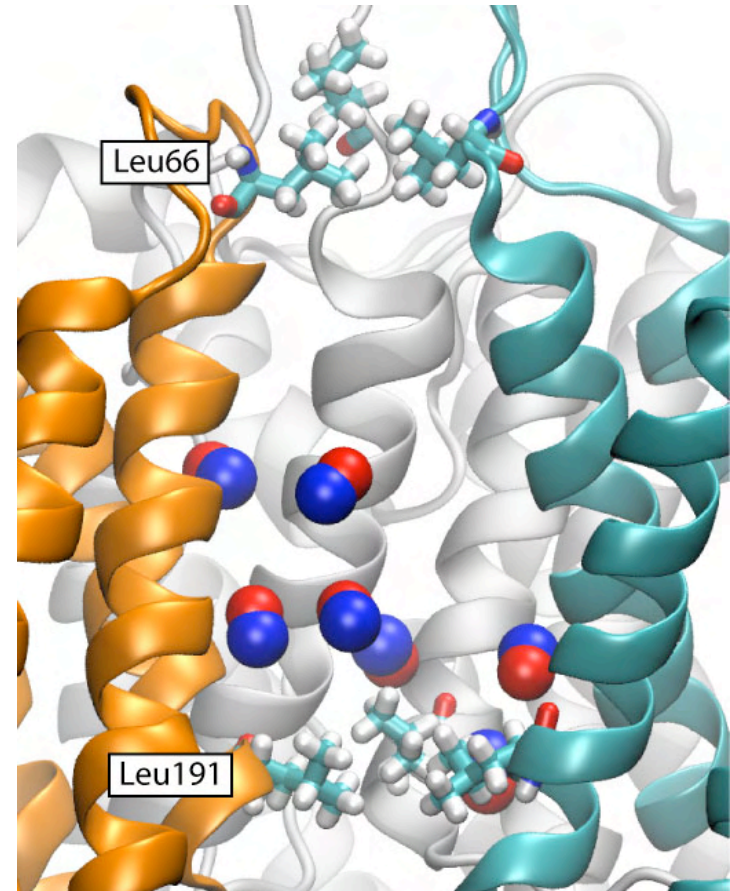
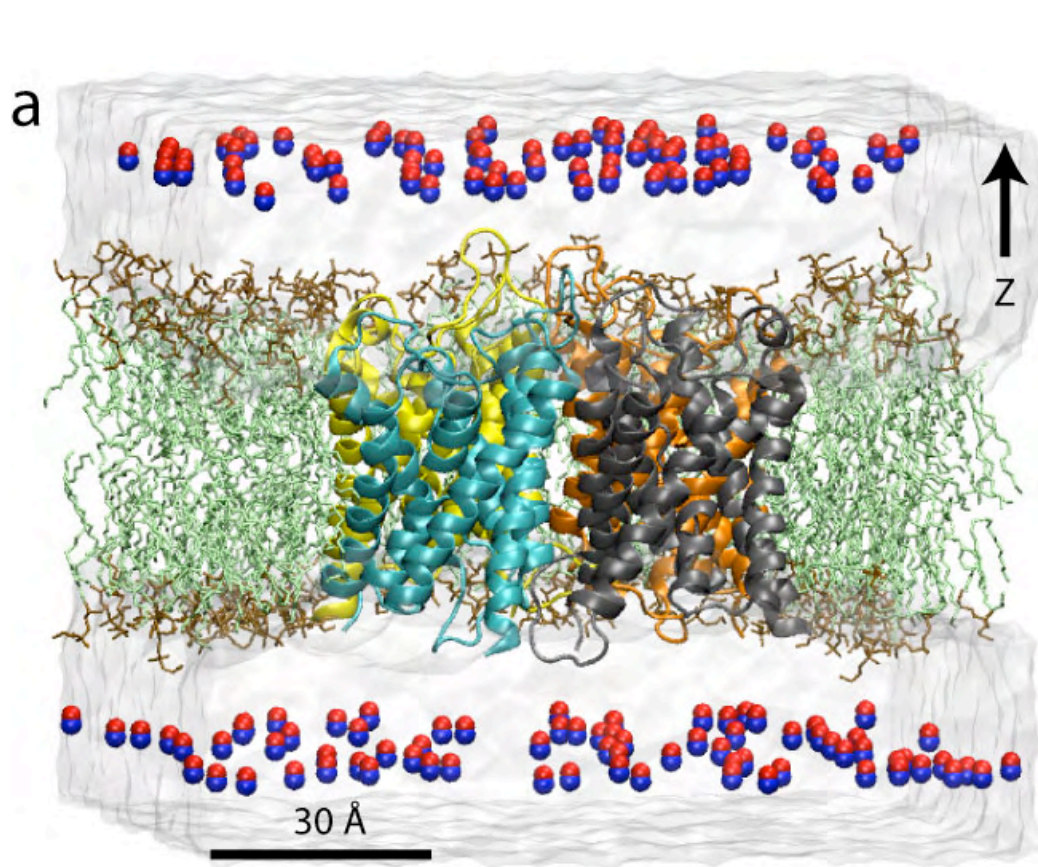
Major Barrier Generated by Structured Water



Barrier identified and characterized through combining the implicit and explicit approaches

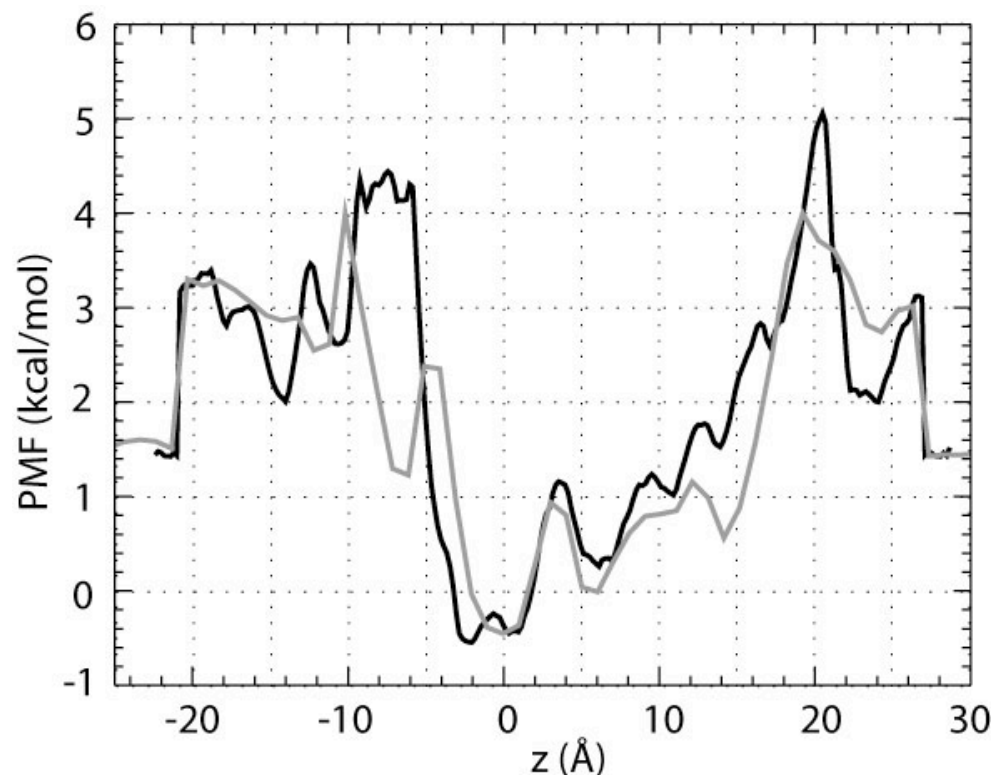
Y. Wang, J. Cohen, W. Boron, K. Schulten, and E. Tajkhorshid, *J. Struct. Biol.*, 2007.
Y. Wang, S. Shaikh, and E. Tajkhorshid, *Physiology*, 2010.

NO• Permeation Through AQP4

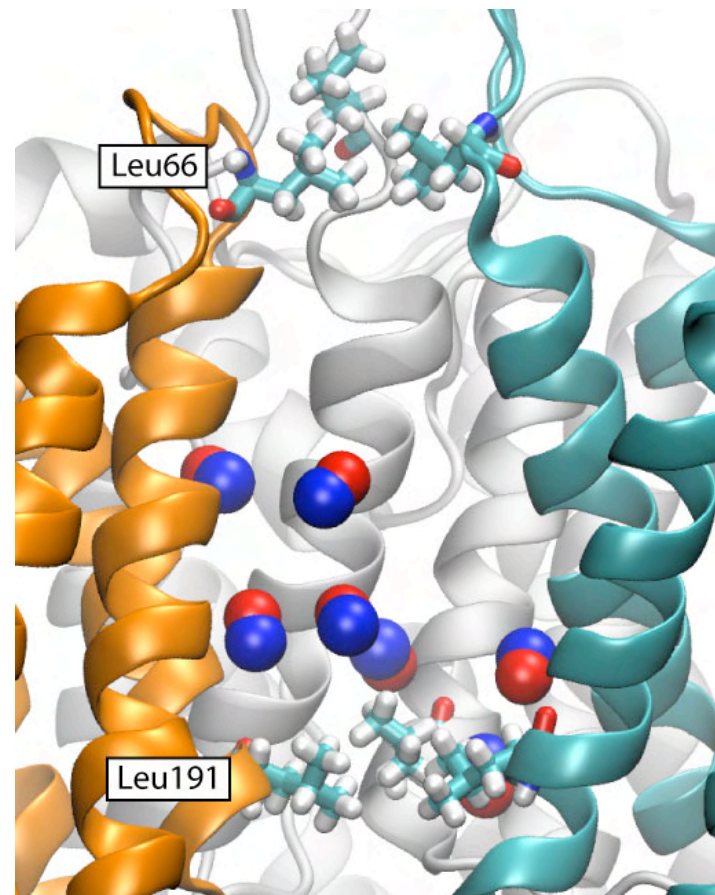


Y. Wang, and E. Tajkhorshid, *Proteins*, 2010.

NO• Permeation Through AQP4



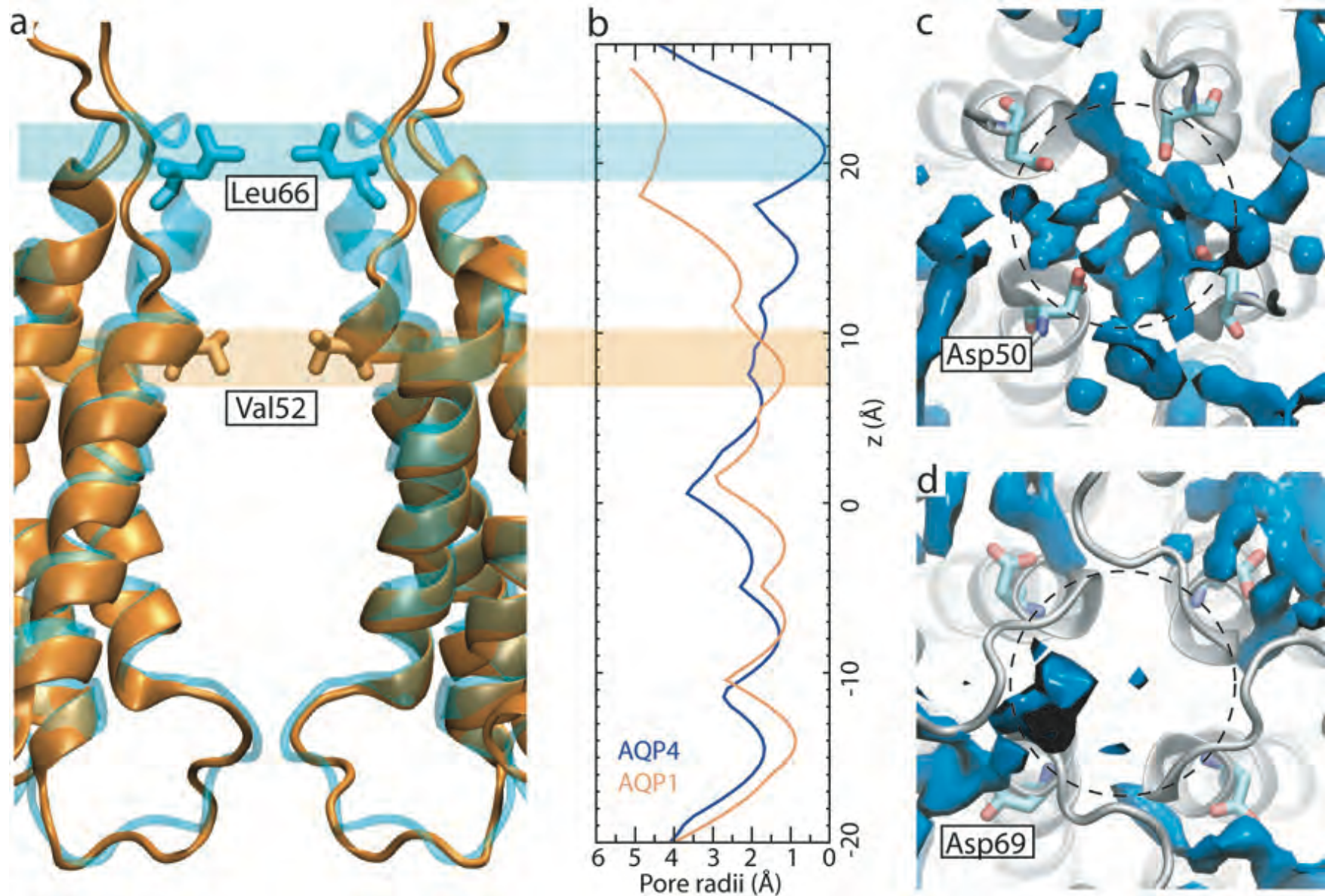
--- Umbrella sampling
--- Implicit sampling



50 ns equilibrium simulation

Y. Wang, and E. Tajkhorshid, *Proteins*, 2010.

Comparison of the Central Pore in AQP1 and AQP4



Gas Transport through Aquaporins

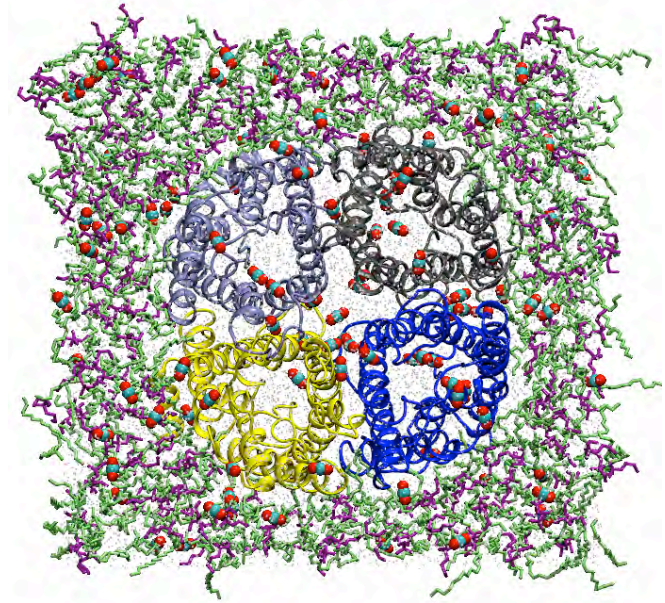
- ◆ Computational evidence for gas transport through a membrane channel

- ◆ Central Pore in AQPs is an optimal pathway for gas diffusion

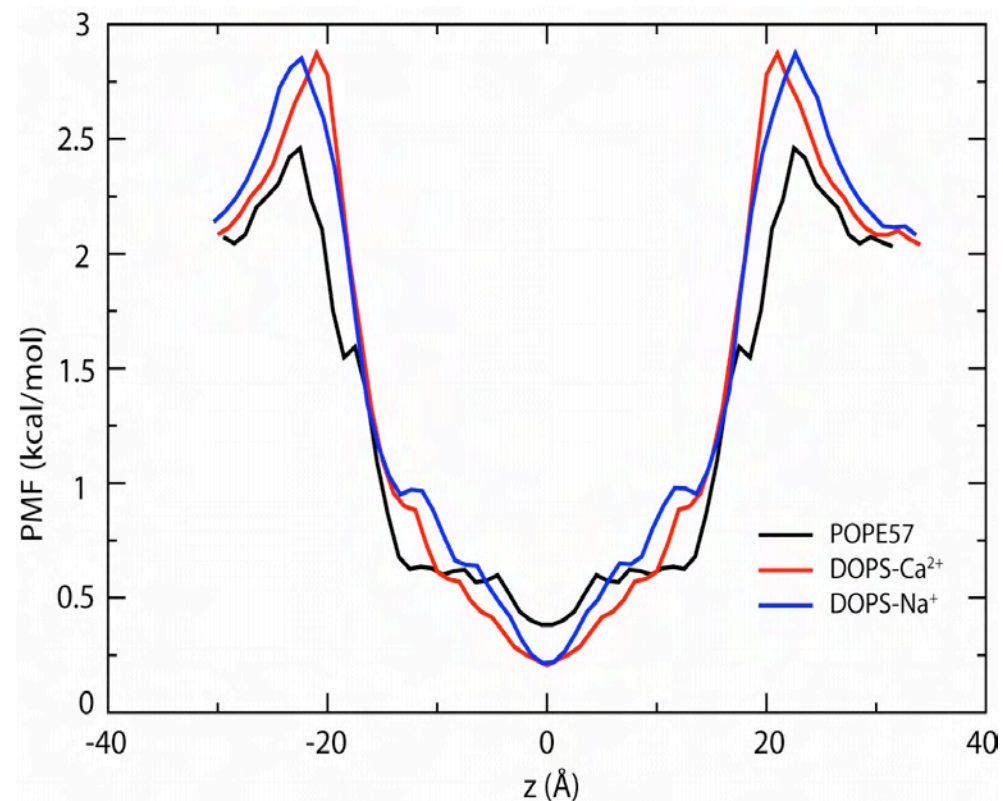
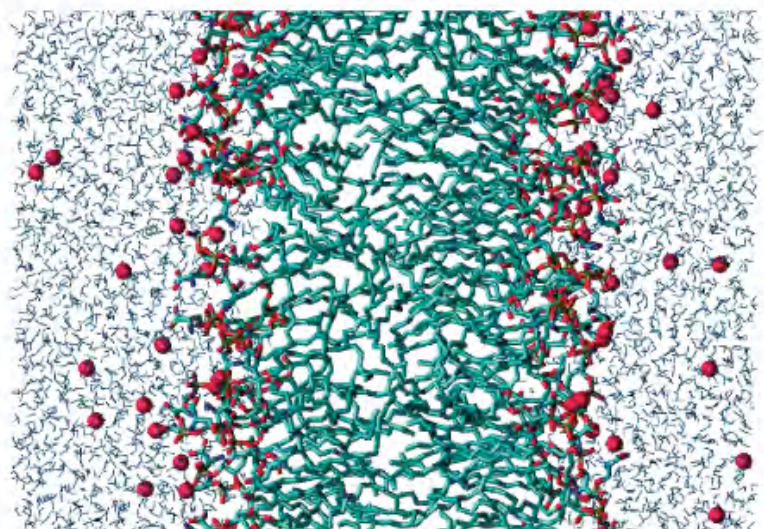
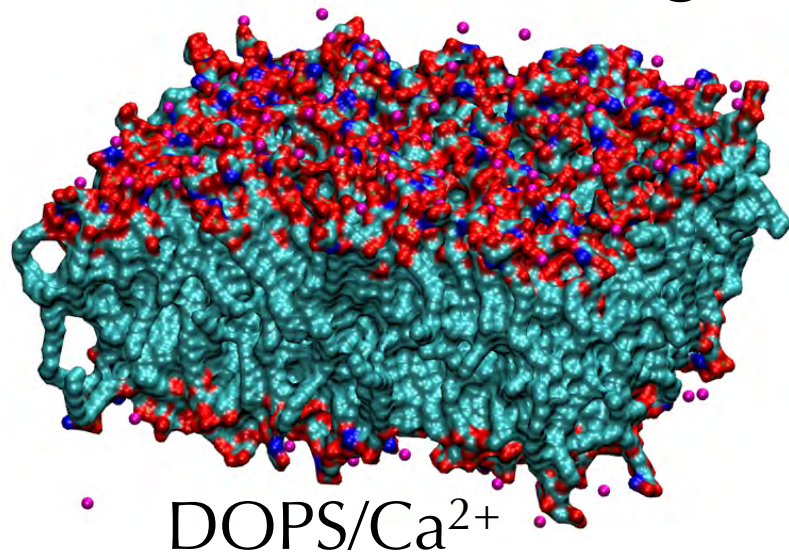
Shared by other oligomeric membrane proteins?

- ◆ AQPs can be physiologically relevant gas channels in lipid bilayer with low gas permeability

- ◆ We can simulate very efficiently the process of gas diffusion, but we rely heavily on reliable initial configurations of lipids/protein



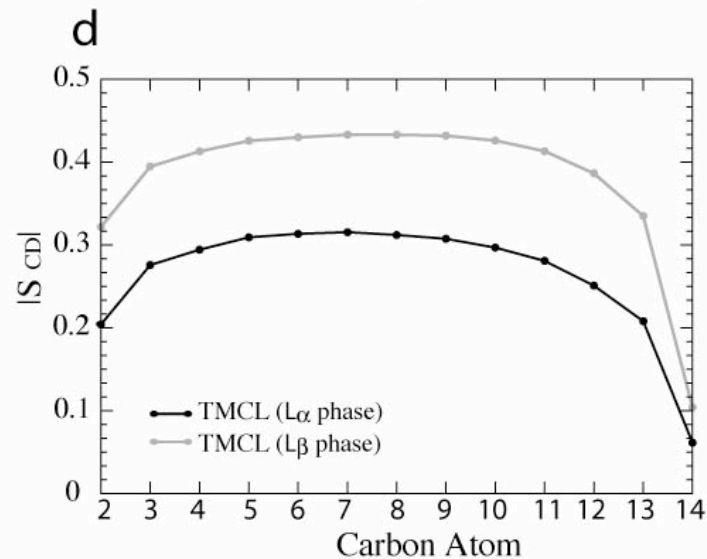
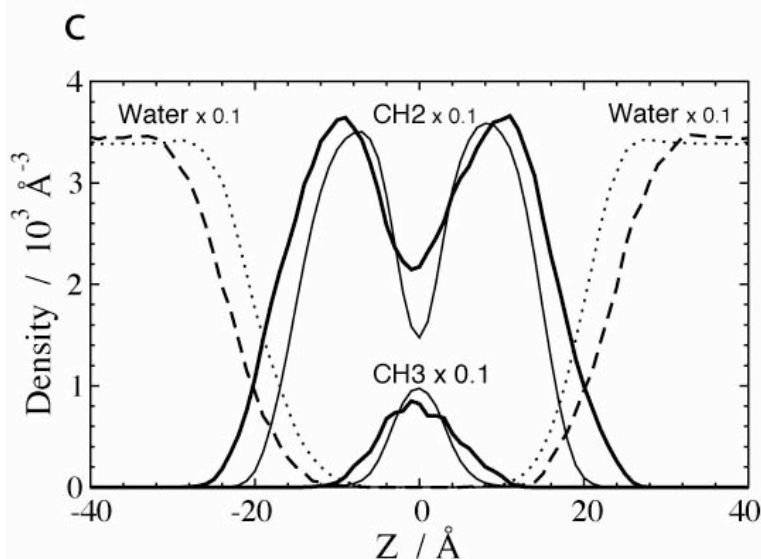
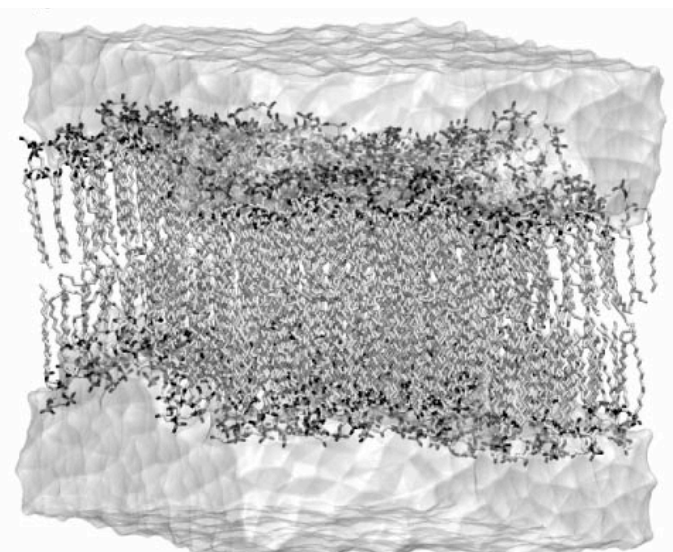
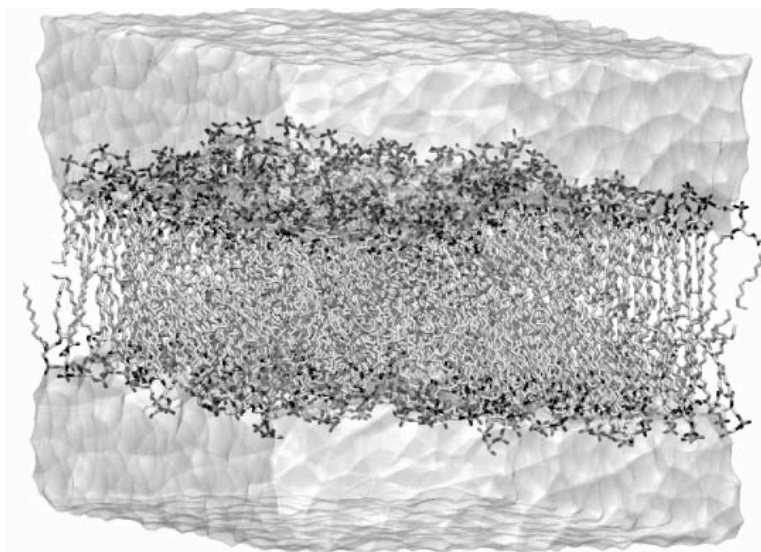
Free Energy of O₂ Permeation Across Charged Lipid Bilayers



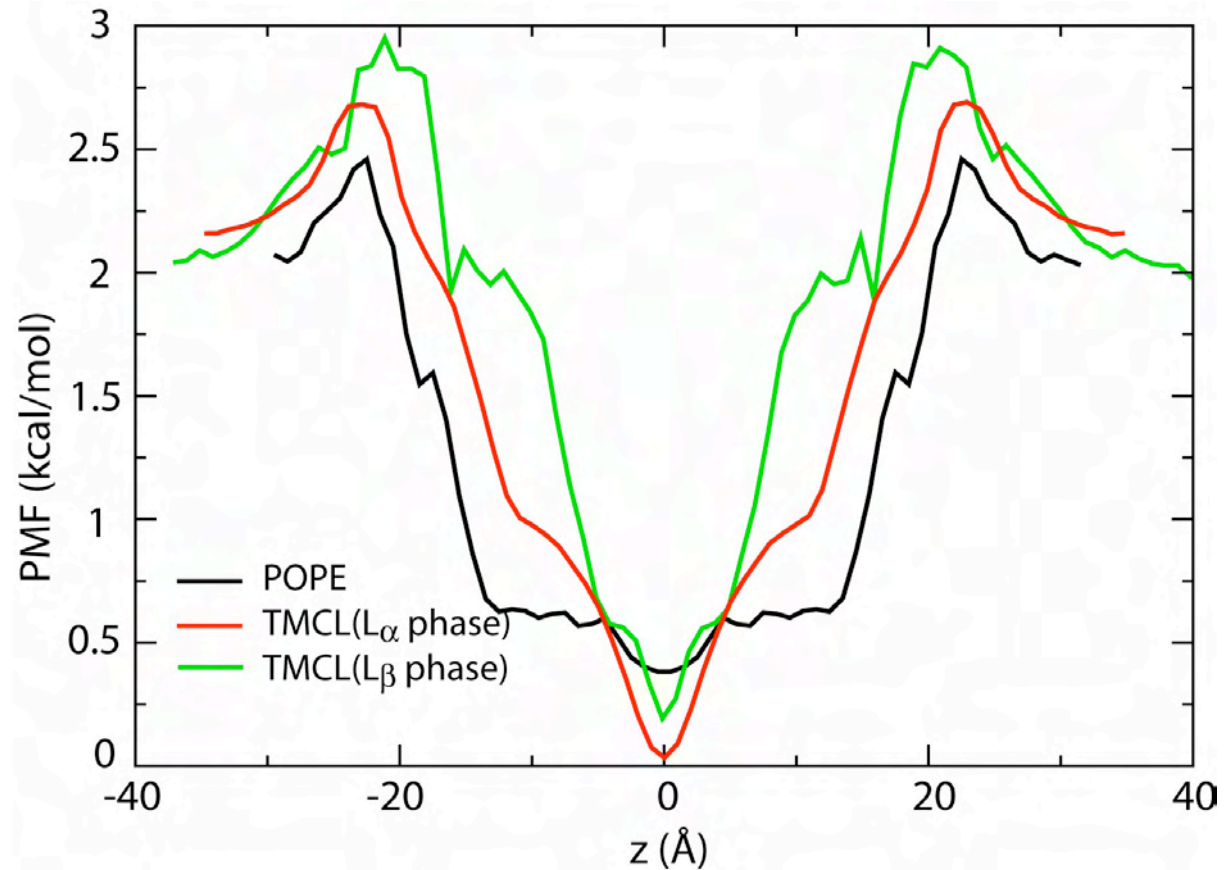
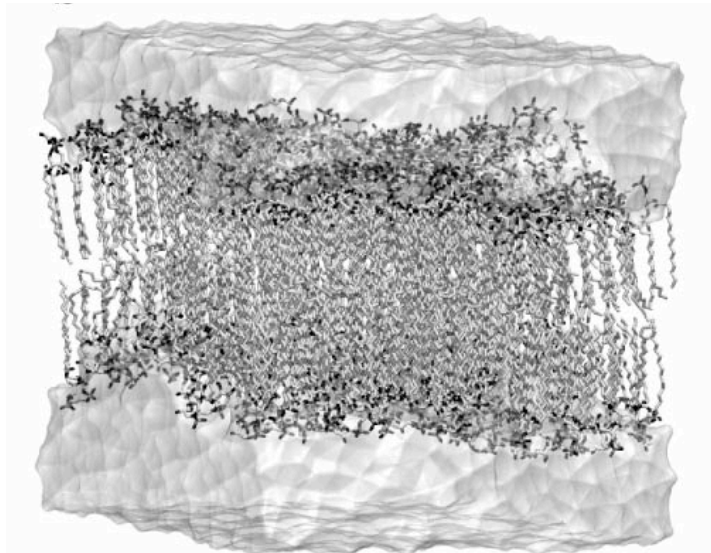
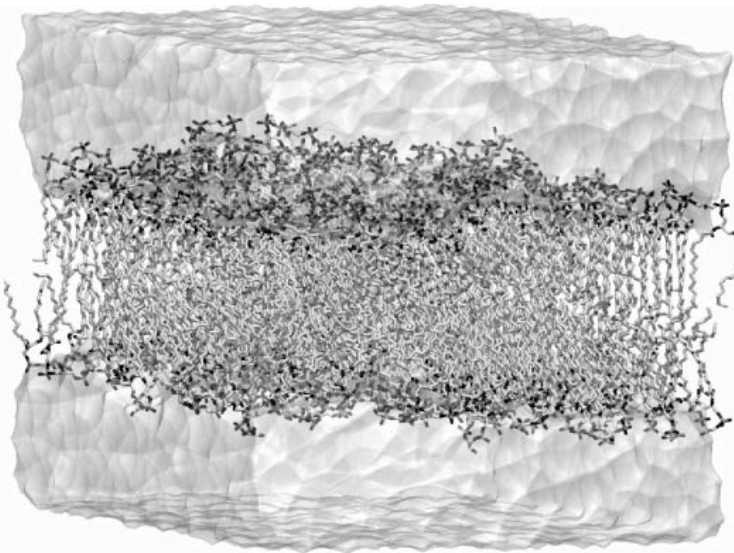
Lipid Phase and Gas Permeation

Liquid phase (30 ns)

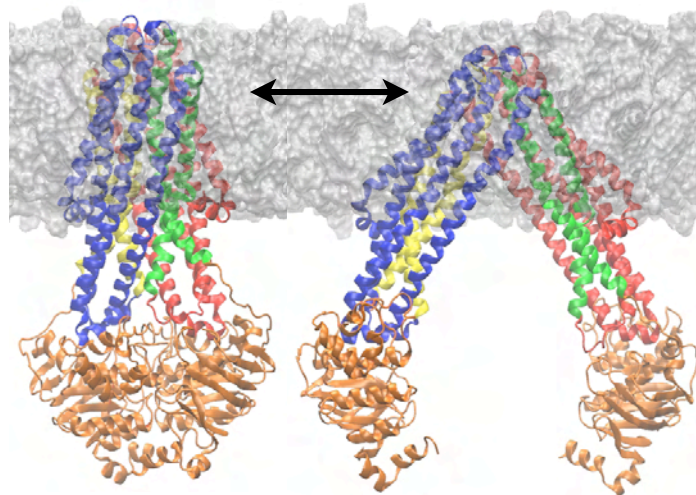
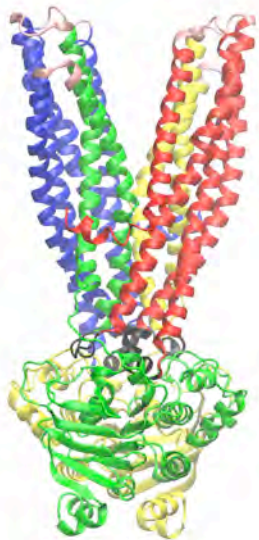
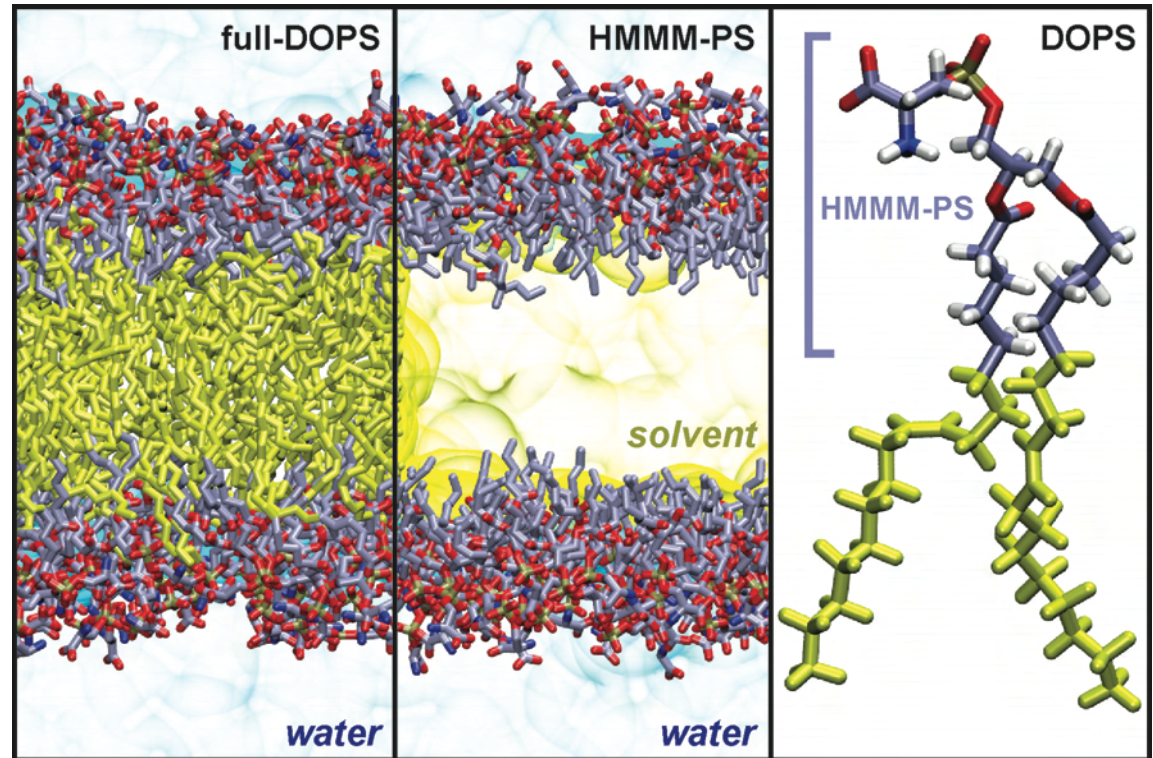
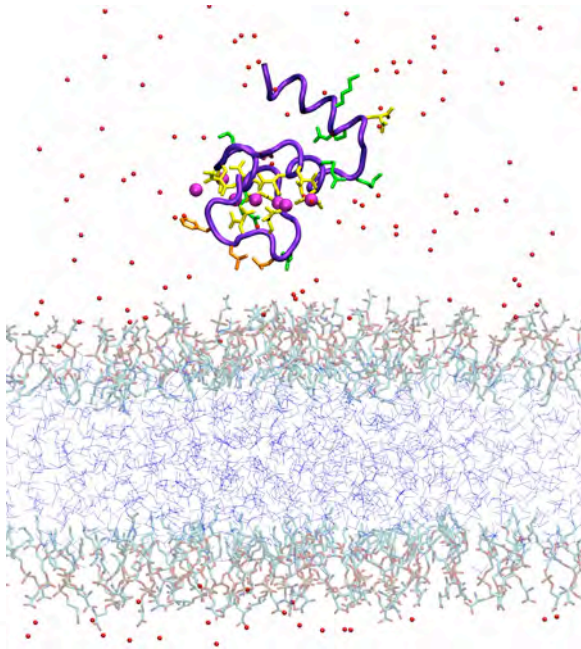
Gel phase (30 ns)



Lipid Phase and Gas Permeation

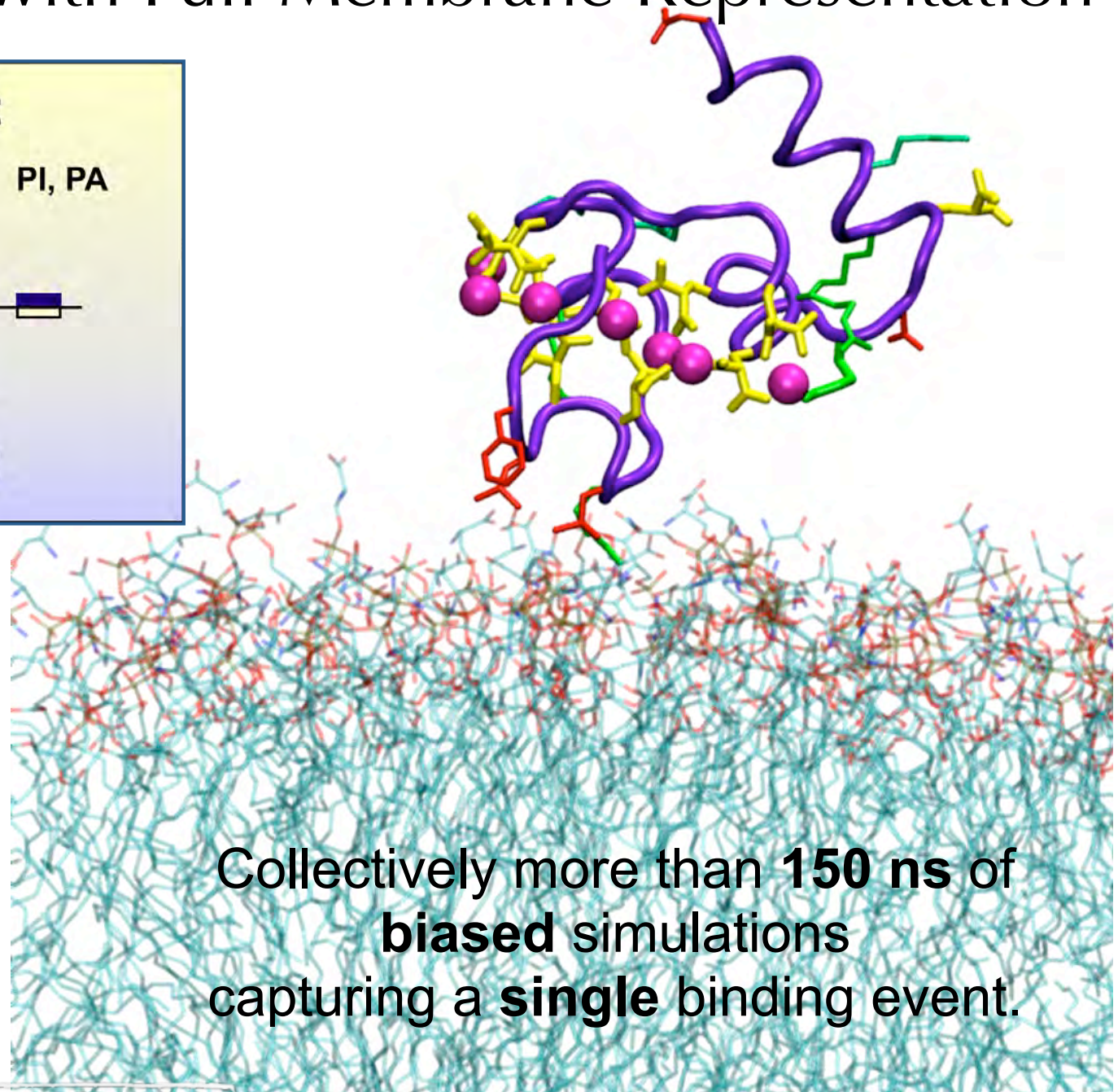
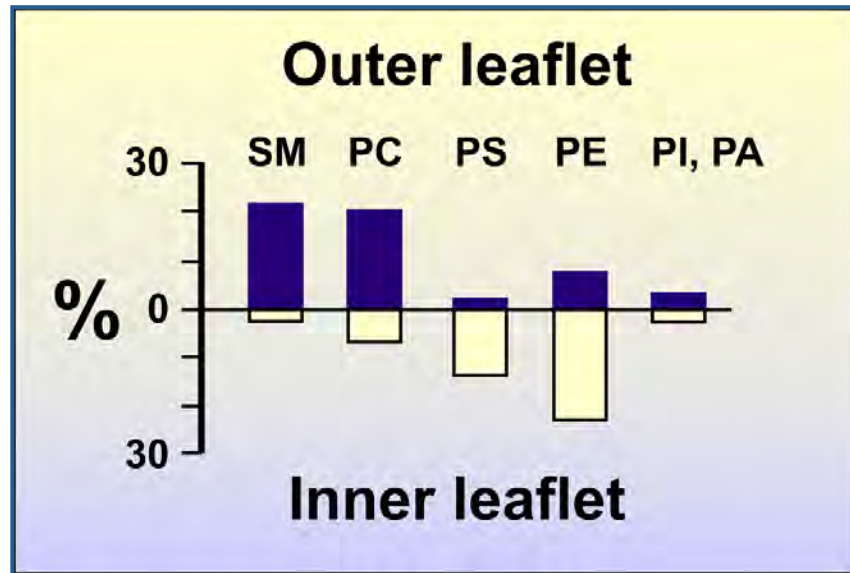


Highly Mobile Membrane Mimetic (HMMM) Model for Membrane Proteins and Phenomena



Ohkubo, Pogorelov, Arcario, Christensen, Tajkhorshid, *Biophysical J.* May 2012.

MD Simulation with Full Membrane Representation



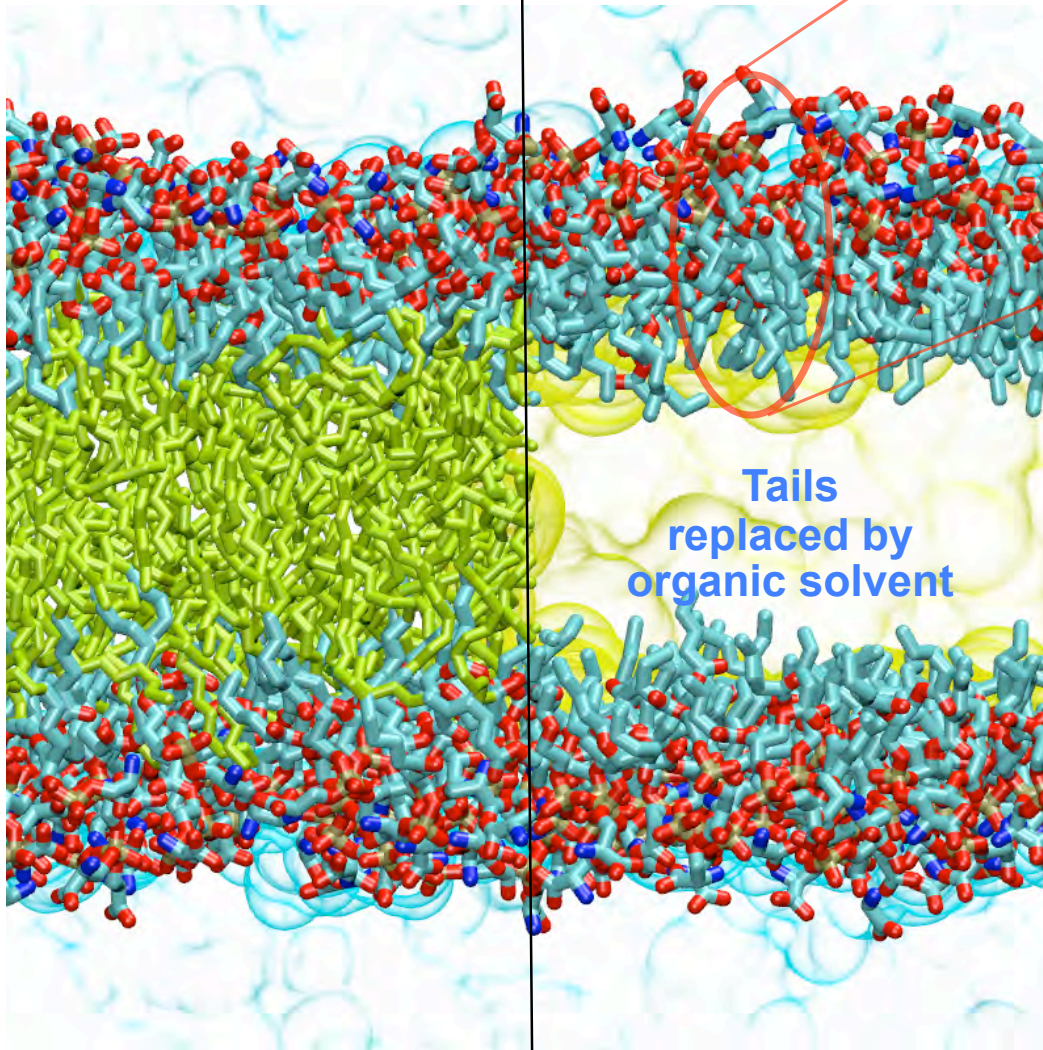
Collectively more than **150 ns** of **biased** simulations capturing a **single** binding event.

HMMM model

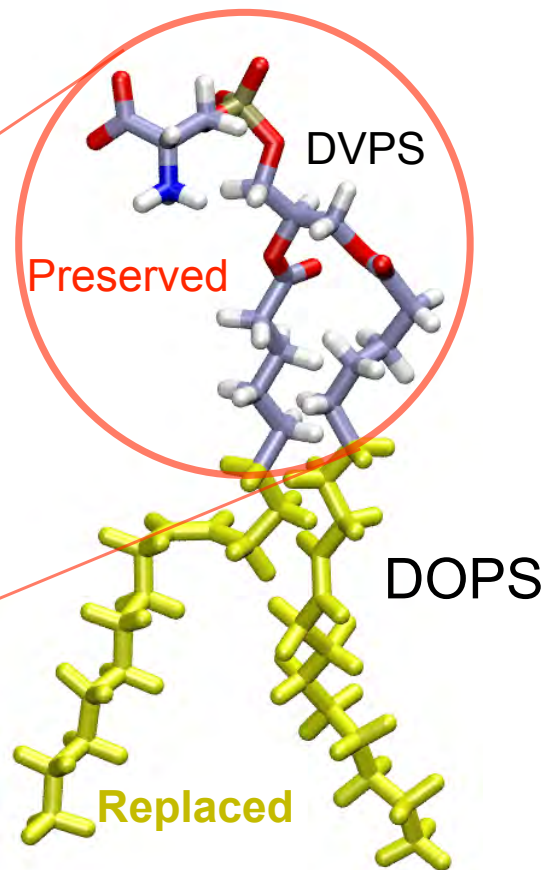
Highly Mobile Membrane Mimetic model

Full model

HMMM model



Tails
replaced by
organic solvent



Advantages

- Increased mobility of lipids
- Retain explicit headgroups allowing for atomic details



Zenmei Ohkubo



Mark Arcario



Taras Pogorelov



Josh Vermaas

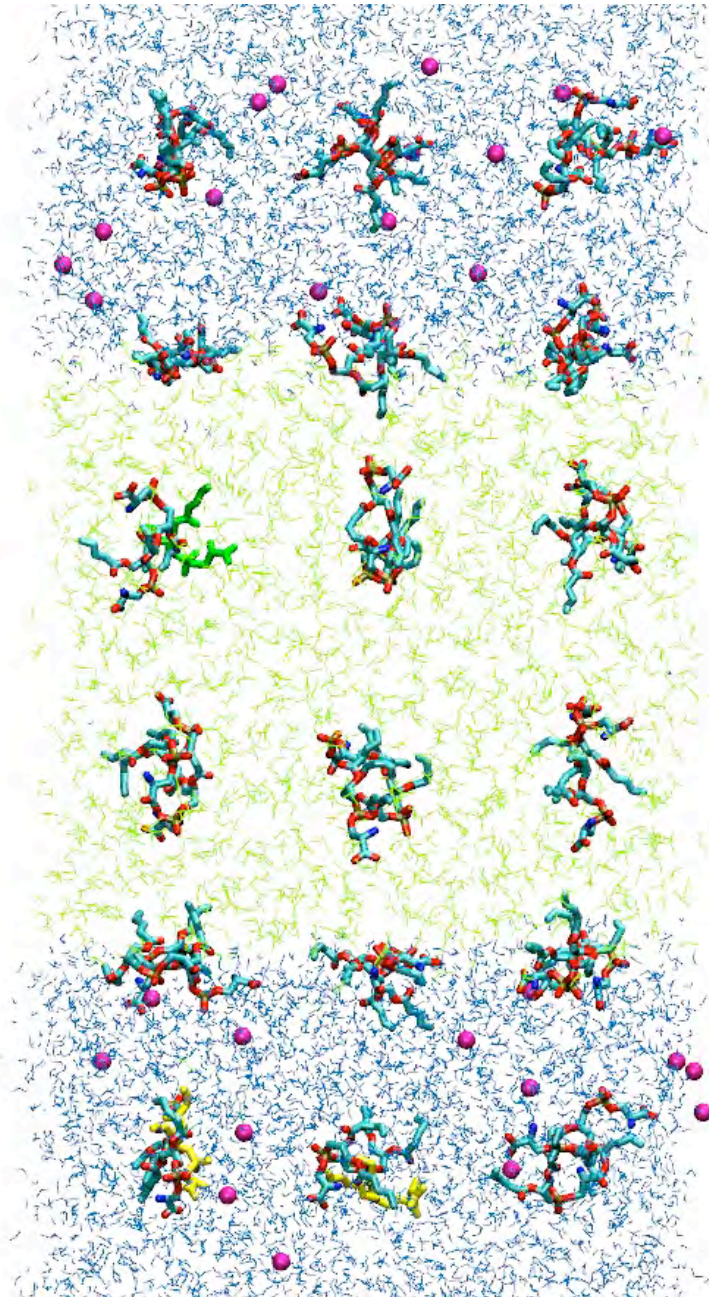
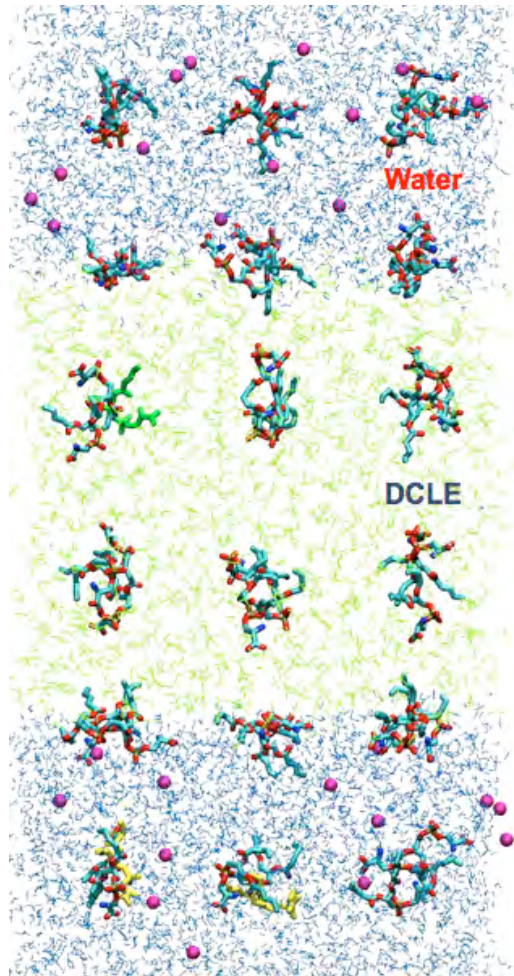


Javier Baylon

Spontaneous and Rapid Formation of a Bilayer



Zenmei Ohkubo

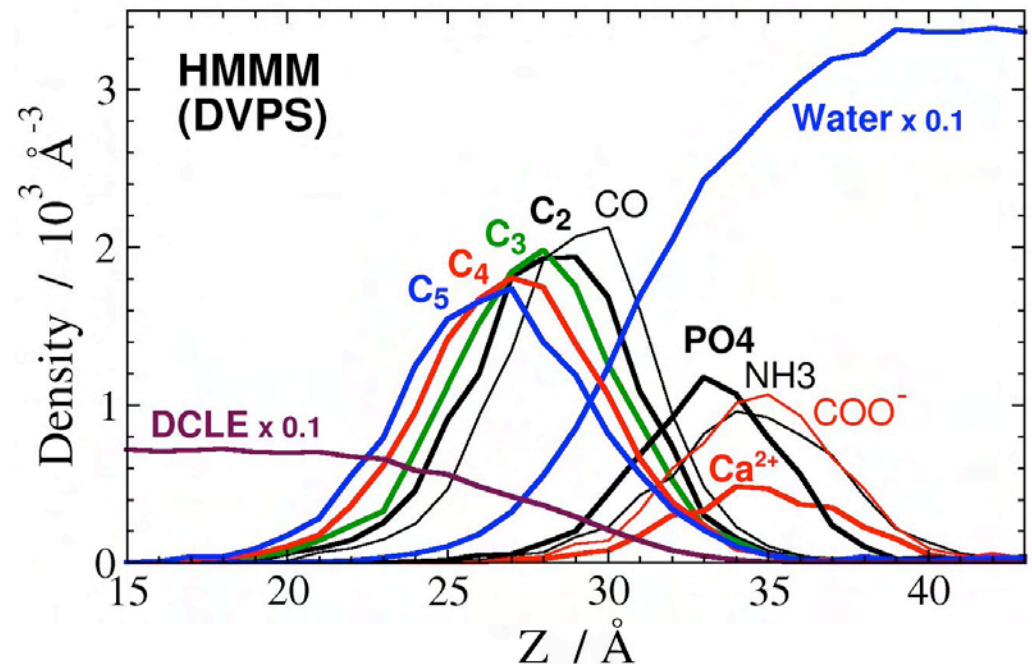
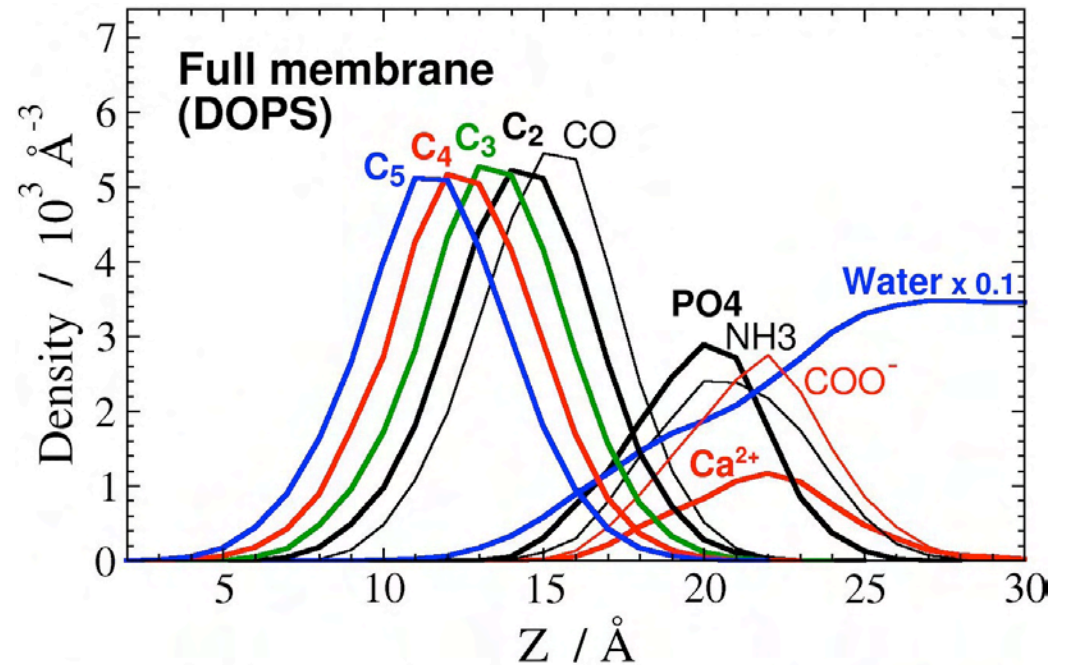
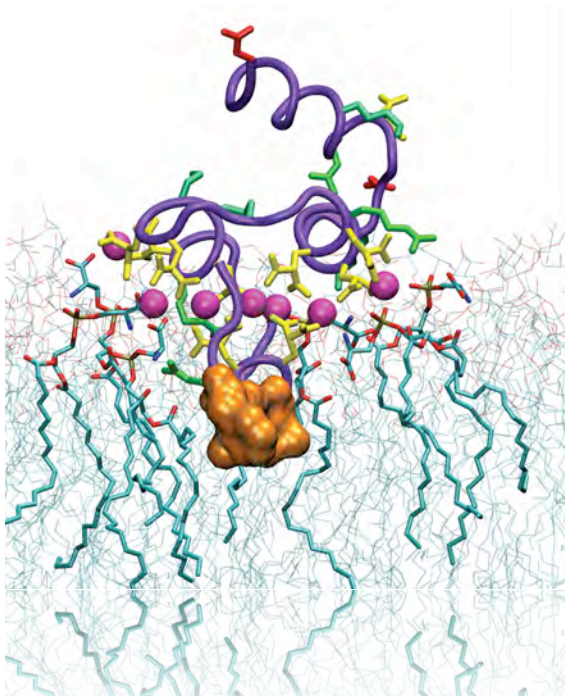


60 x 60 x 120 Å
DVPSs at 3 x 3 x 6 grid points
(22 ns)

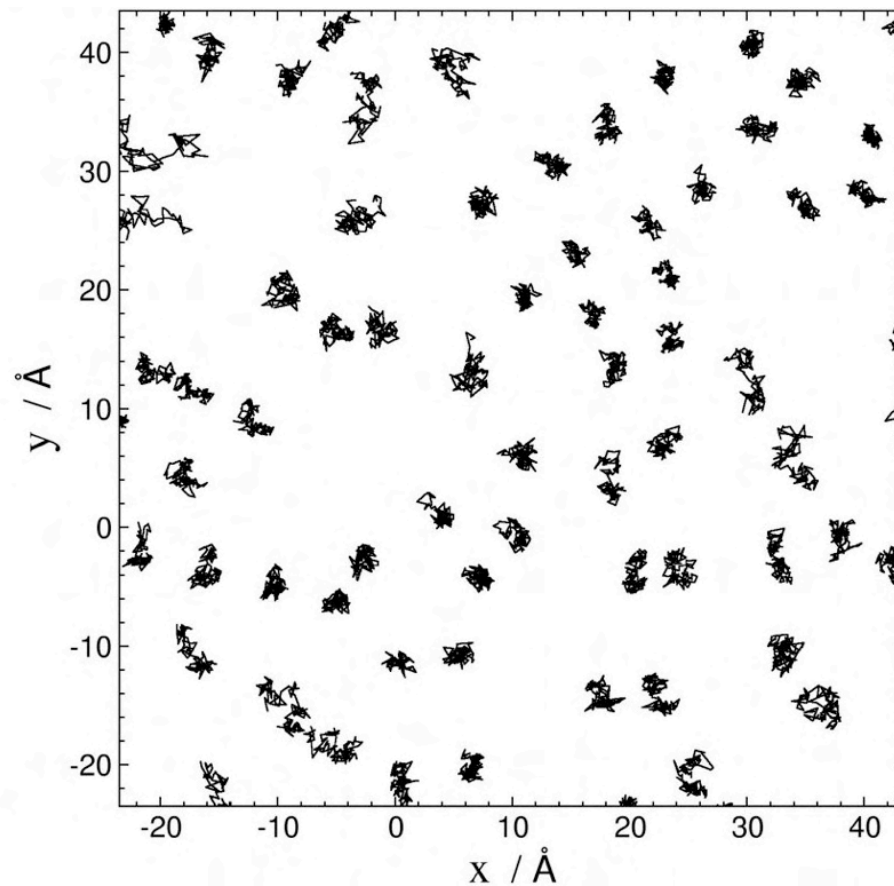
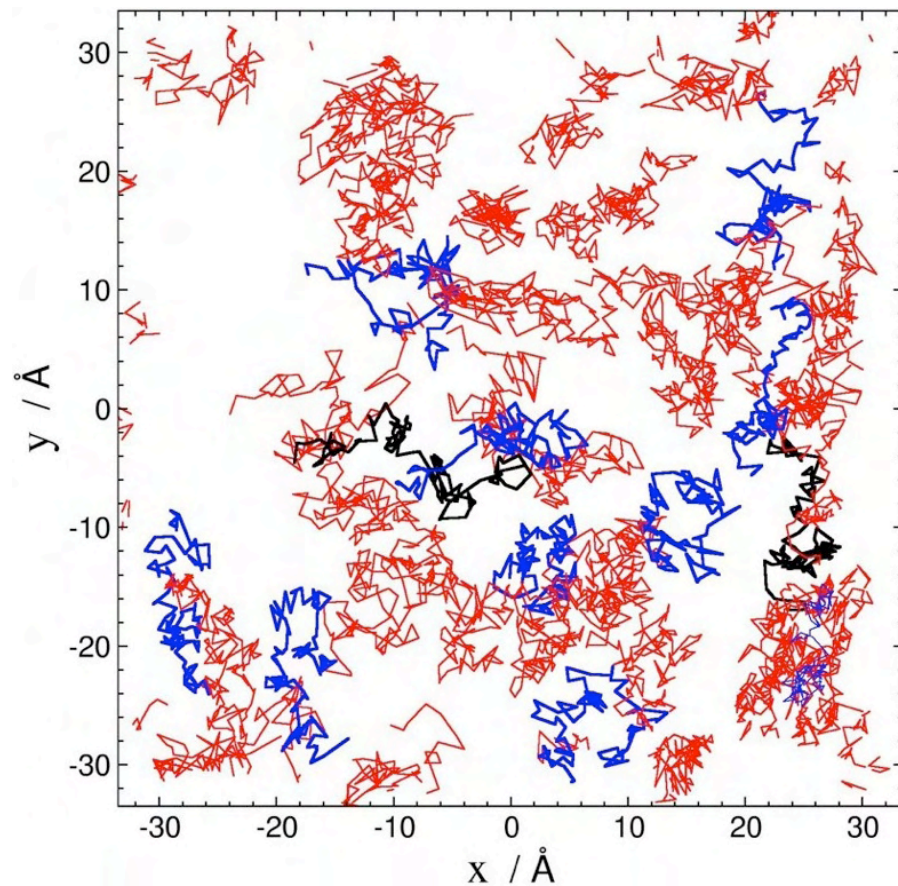
HMMM- Preserving the “Face” of the Lipid Bilayer

Perfect match in the membrane profile particularly in the head group region

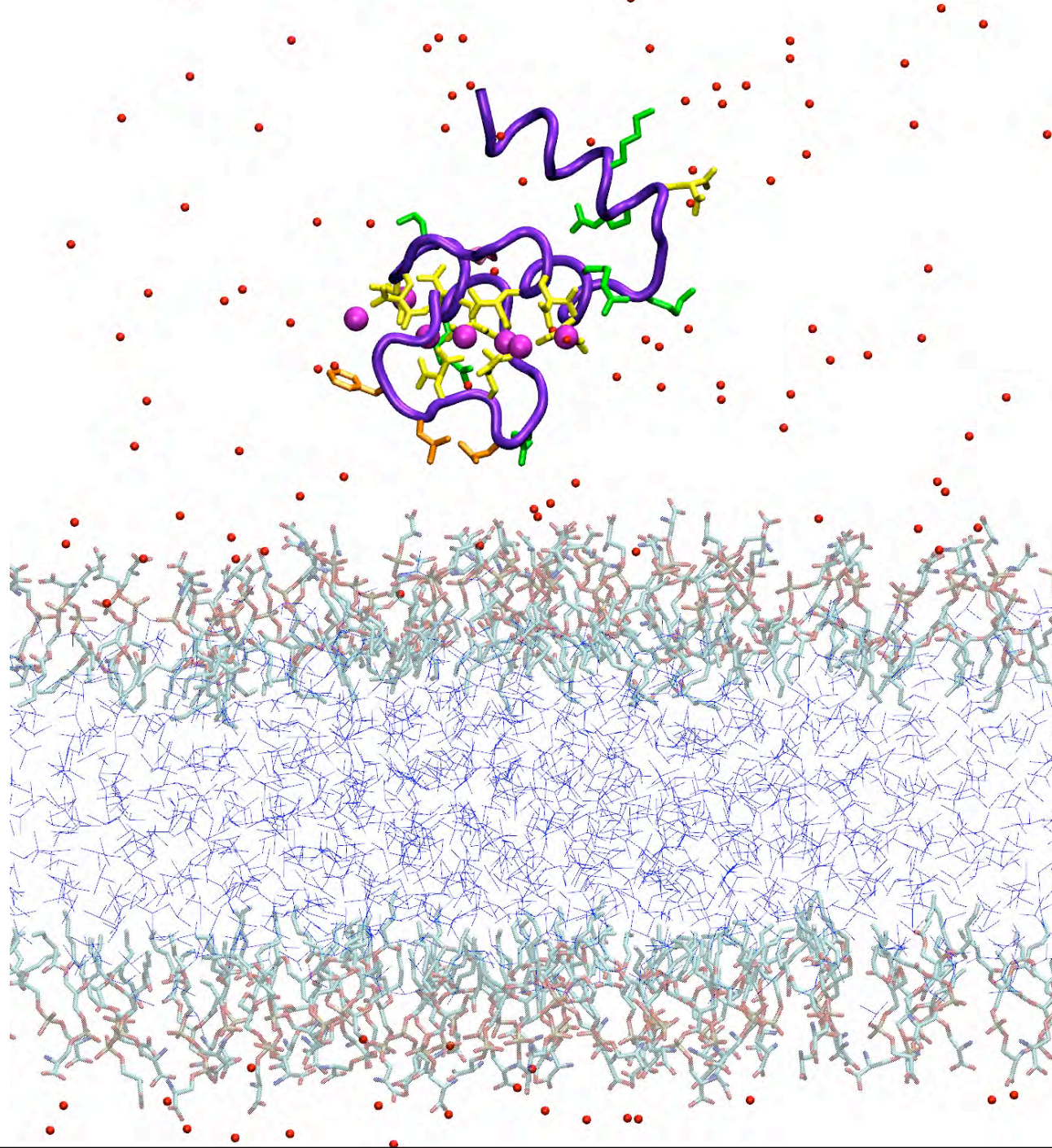
Critical for proper description of lipid protein interactions



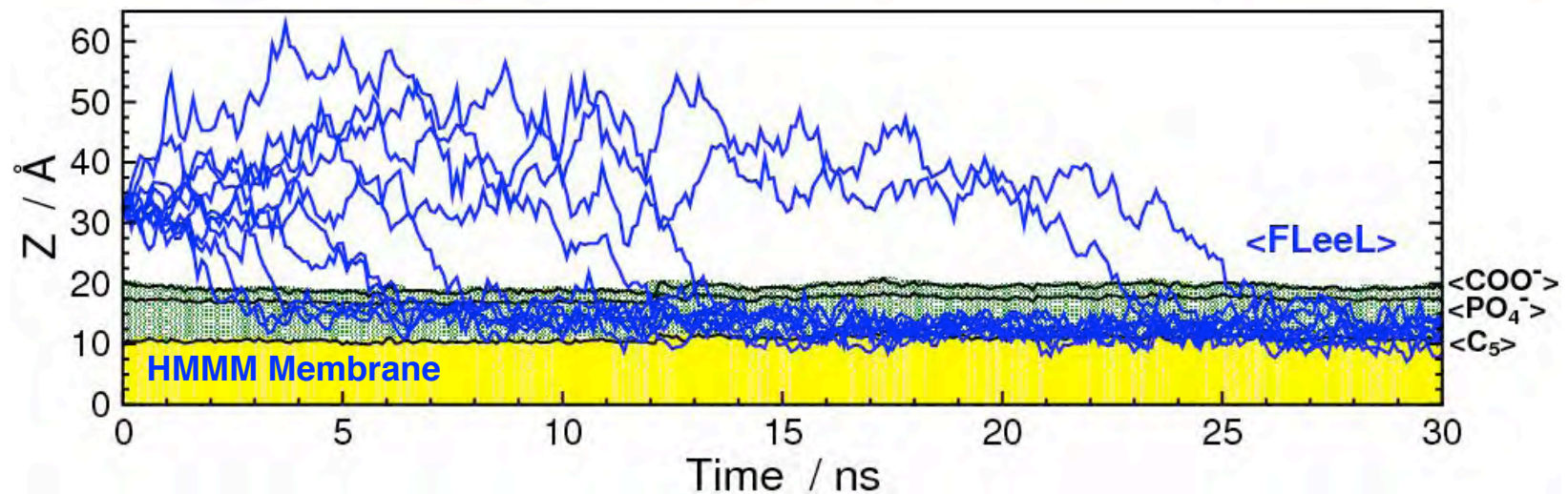
HMMM – lipids are more mobile than full-lipids



Spontaneous Insertion of FVII-GLA



Spontaneous Membrane Binding ($n = 10$)

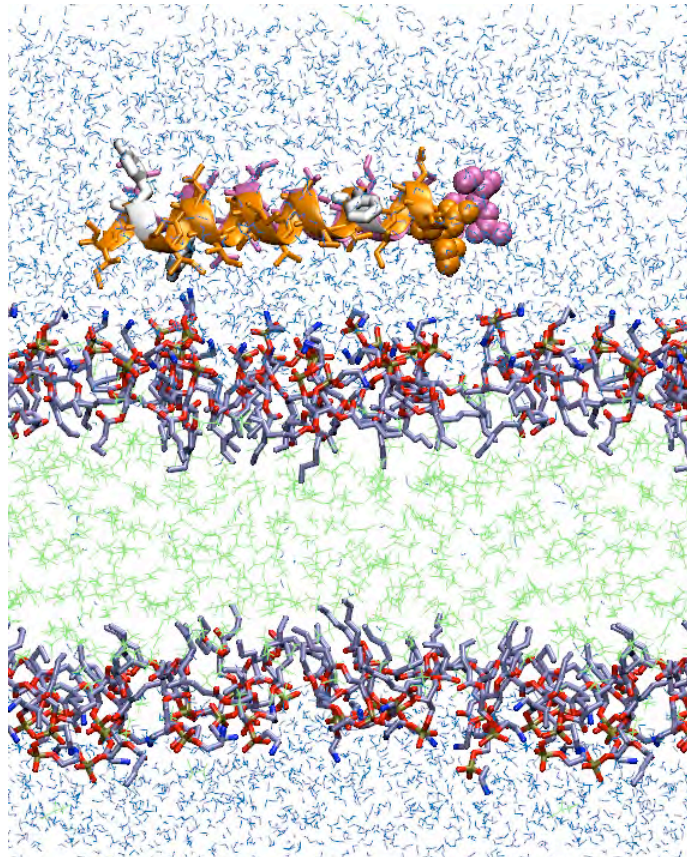


Spontaneous Insertion of Transmembrane Helices



Taras Pogorelov

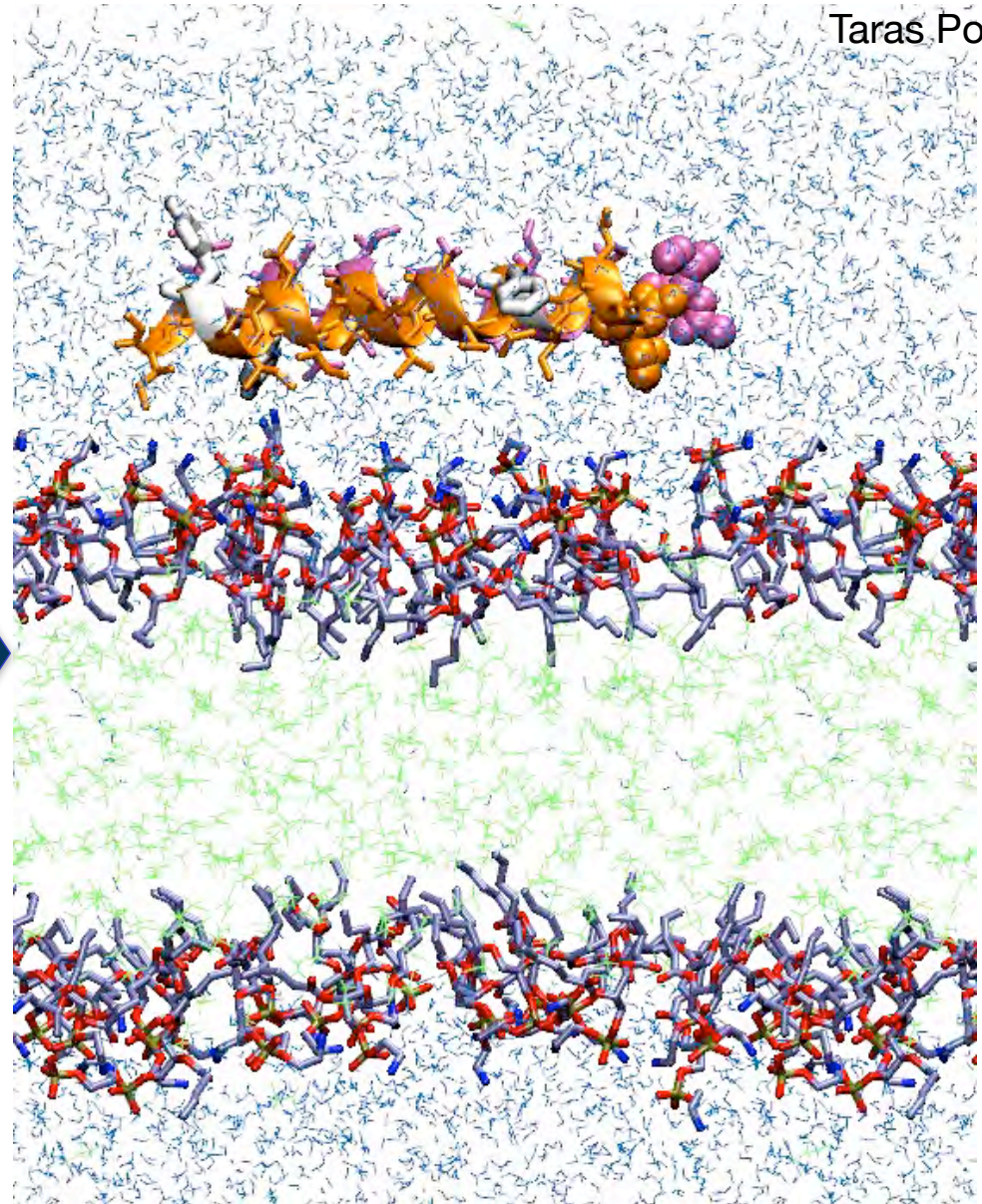
t = 0



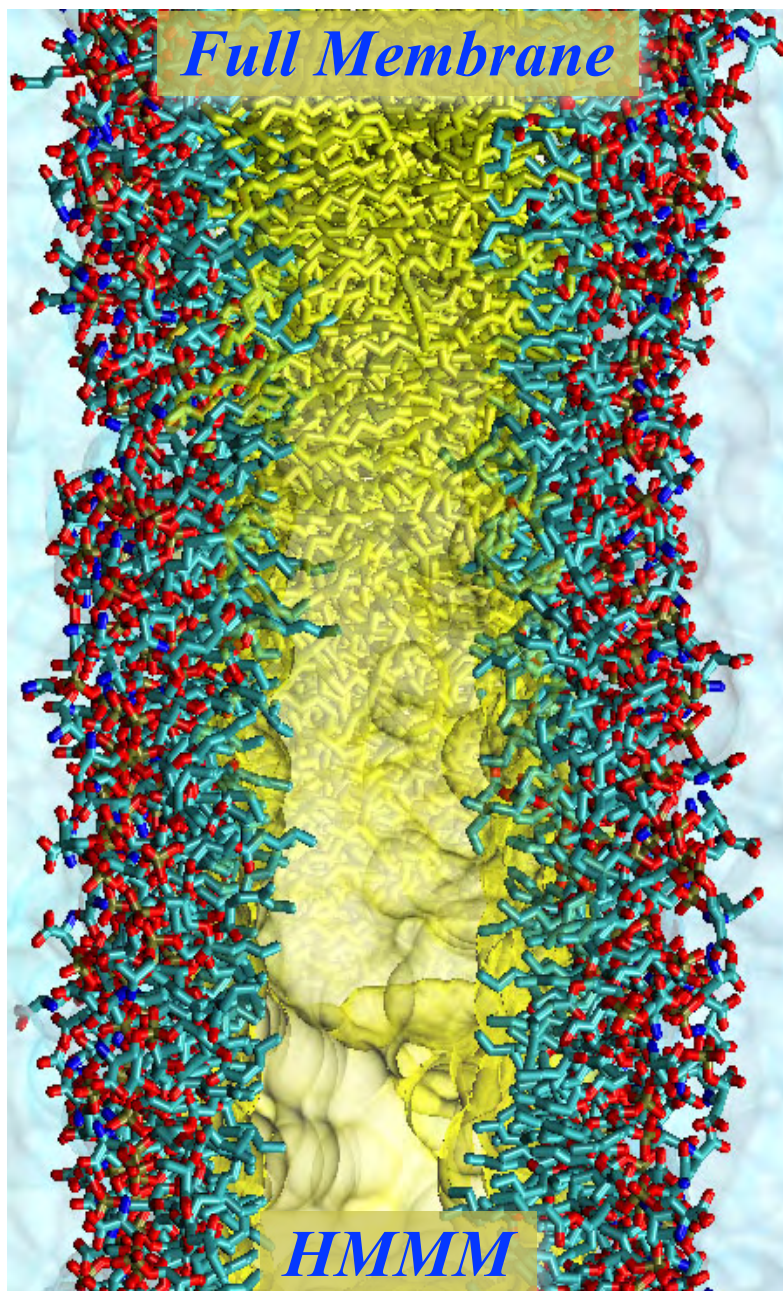
50 x 50 x 75 Å

Glycophorin A monomers: 2
z-constraint on 2 carbonyl carbons

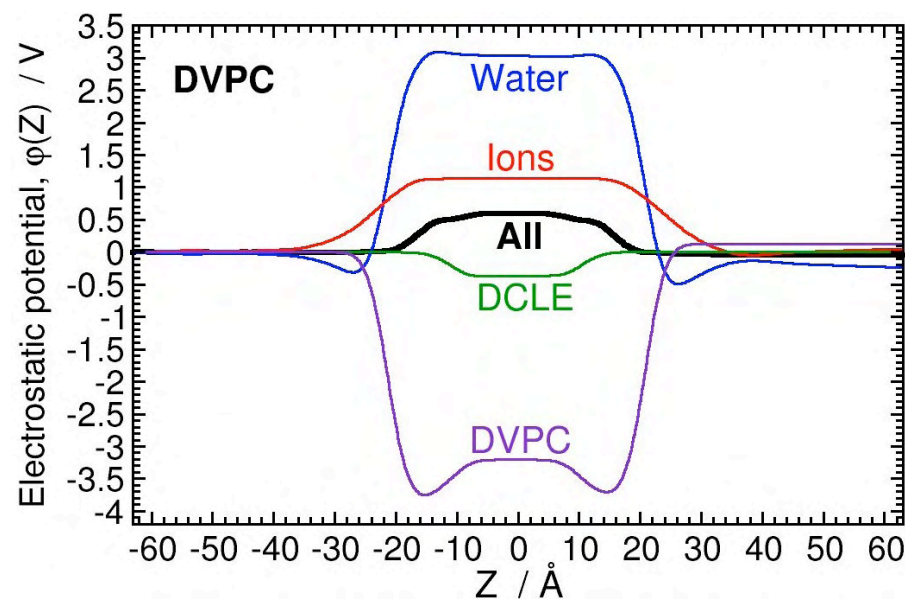
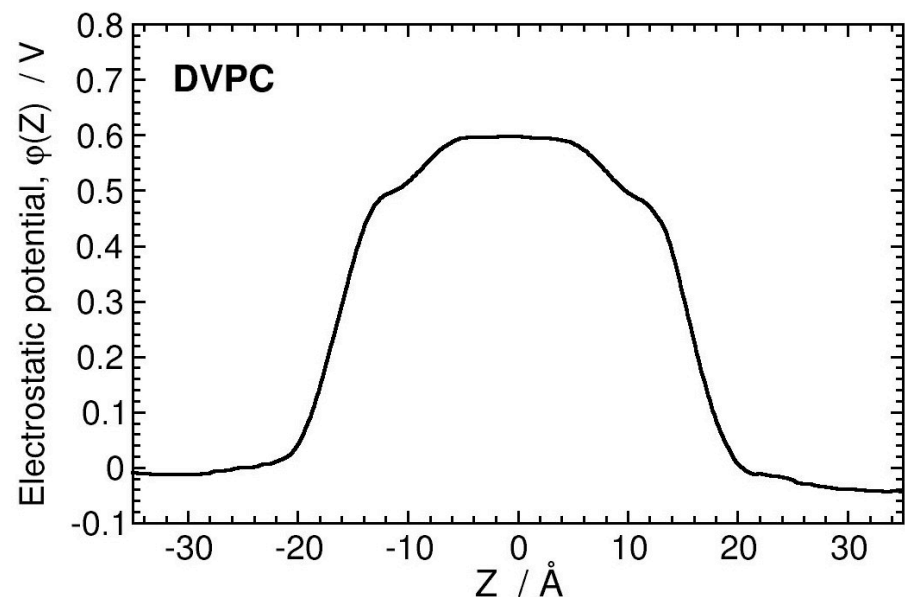
12 ns



Quantitative Characterization and Optimization of HMMM

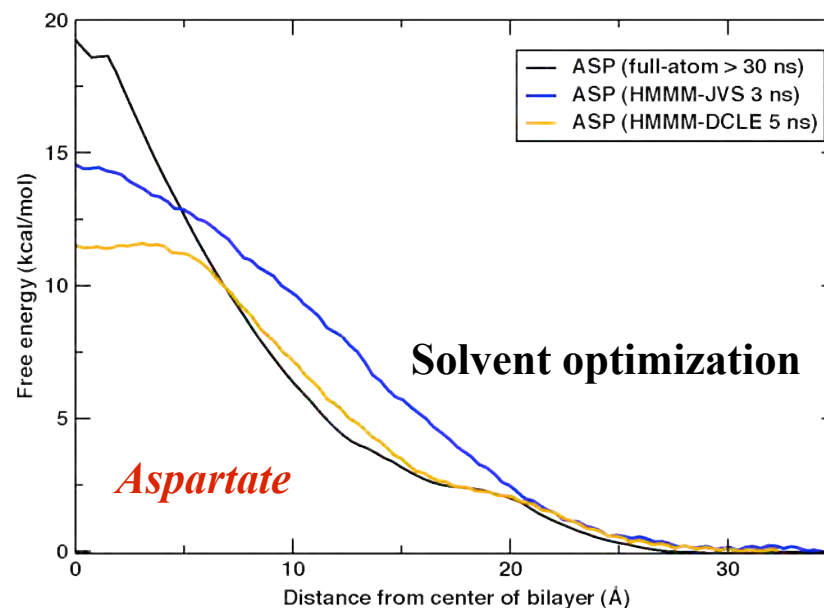
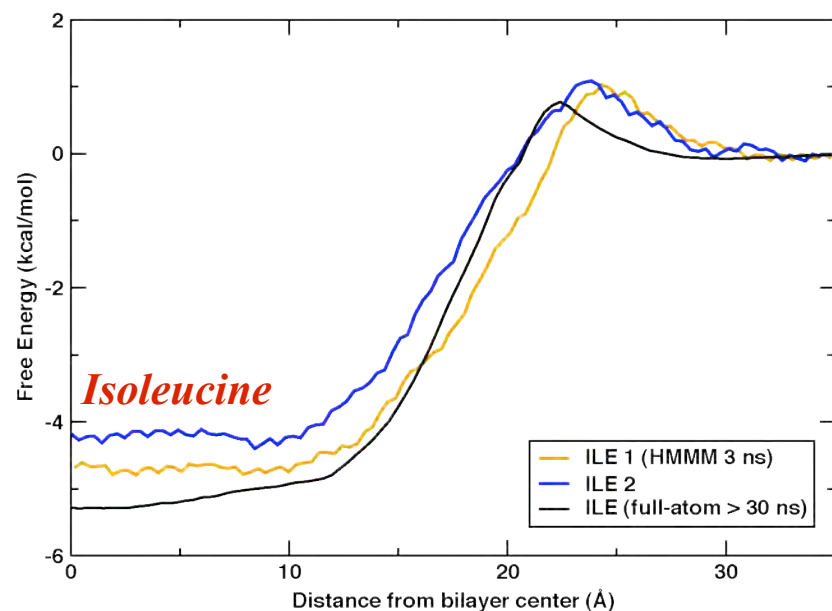
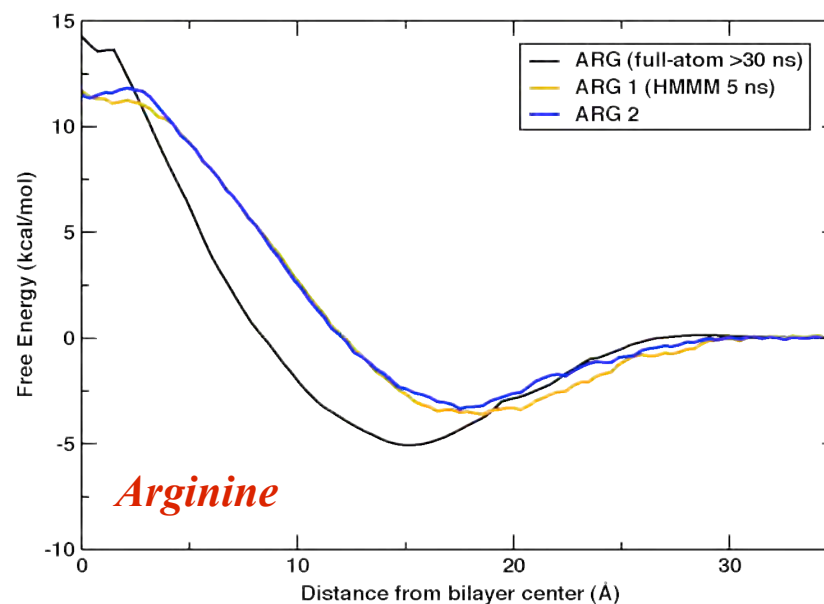
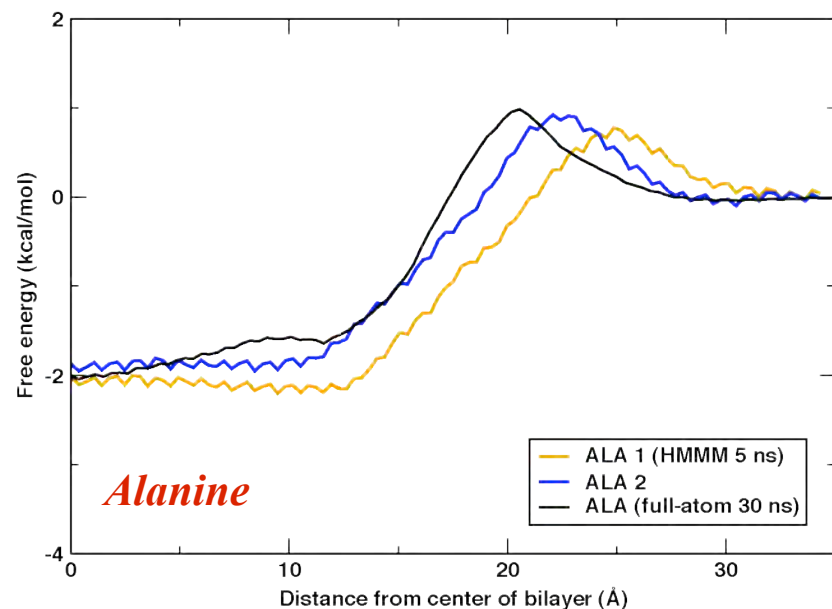


Membrane Dipolar Potential



Quantitative Characterization and Optimization of HMMM

PMF of Amino Acid Insertion

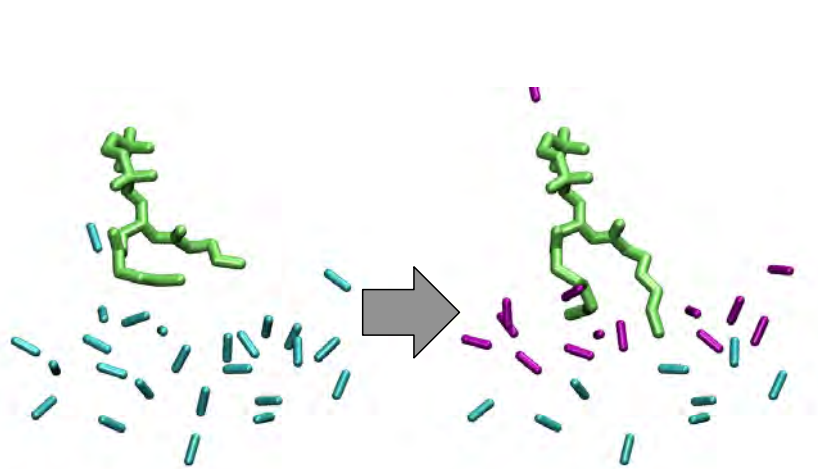


* Black lines: Full membrane data from Biophys. J. 94, 3393, 2008.

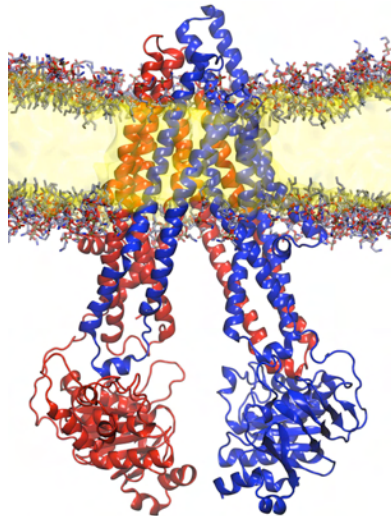
Tail-Gro - Stepwise transformation of HMMM to full membrane representation



Josh Vermaas

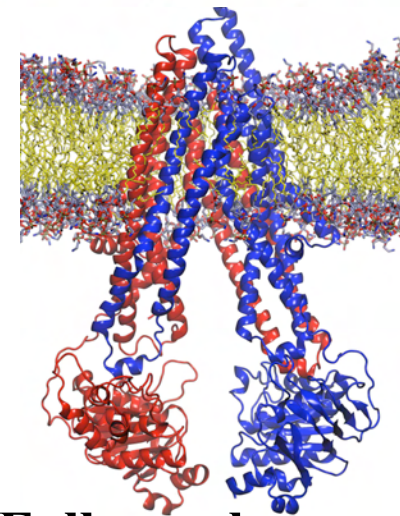
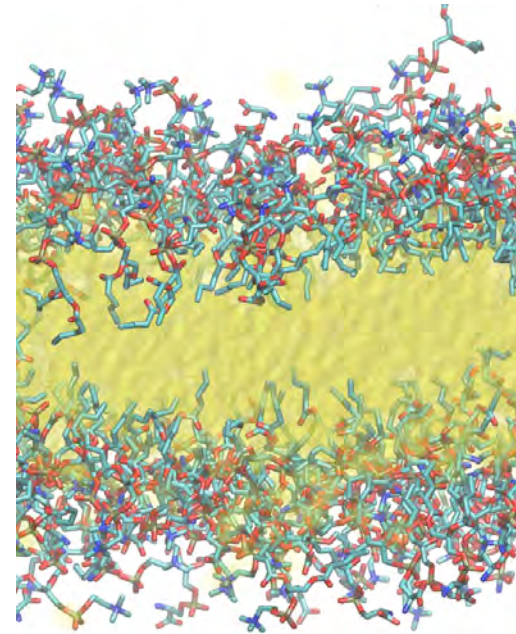


**Step-wise insertion
of P-glycoprotein**



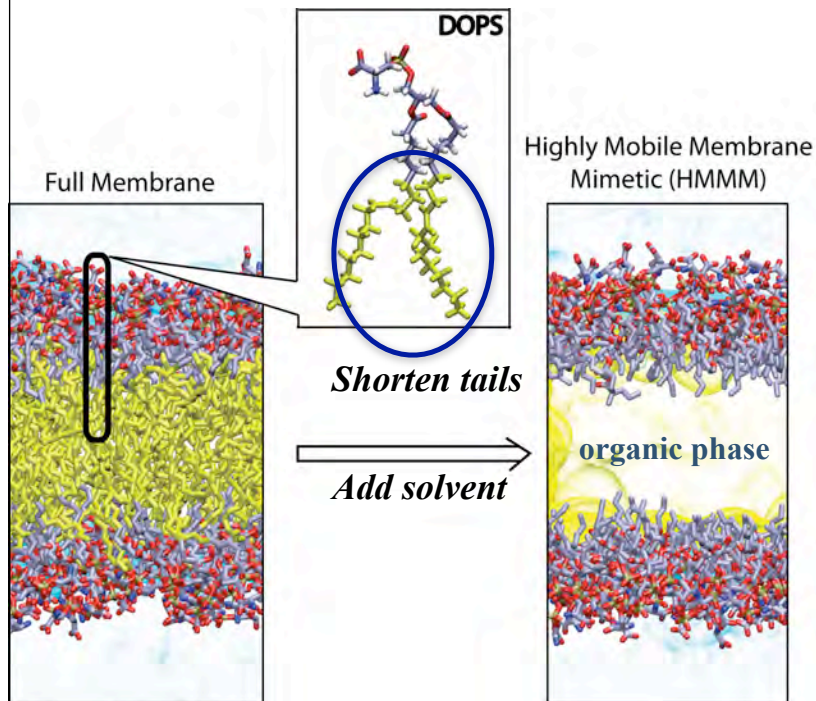
HMMM

Grow tails

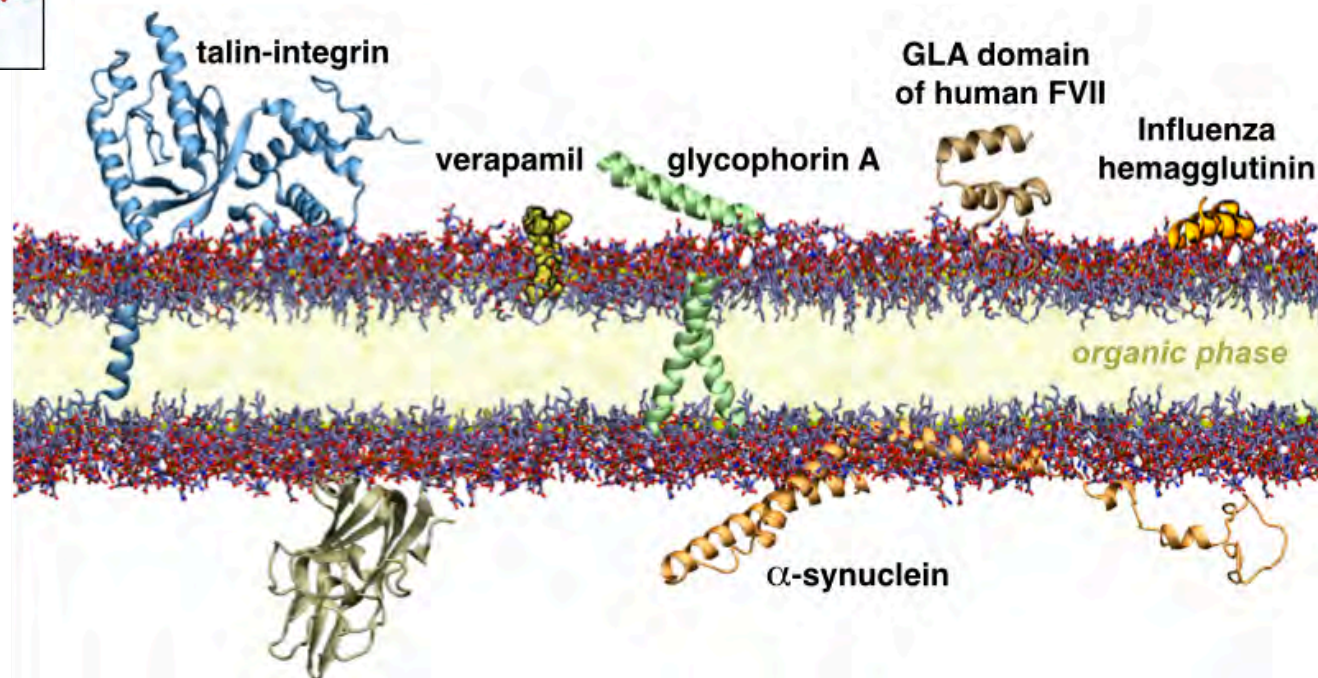
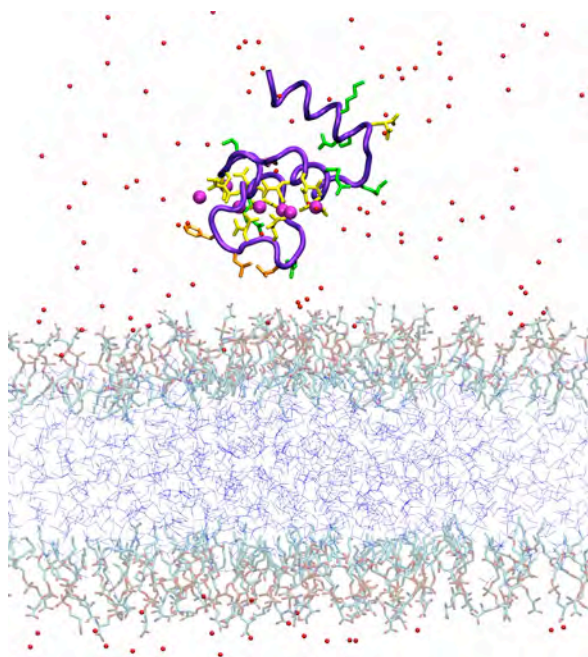


Full membrane

Highly Mobile Membrane Mimetic Model (HMMM)



Facilitating dynamical studies
of membrane-associated
phenomena



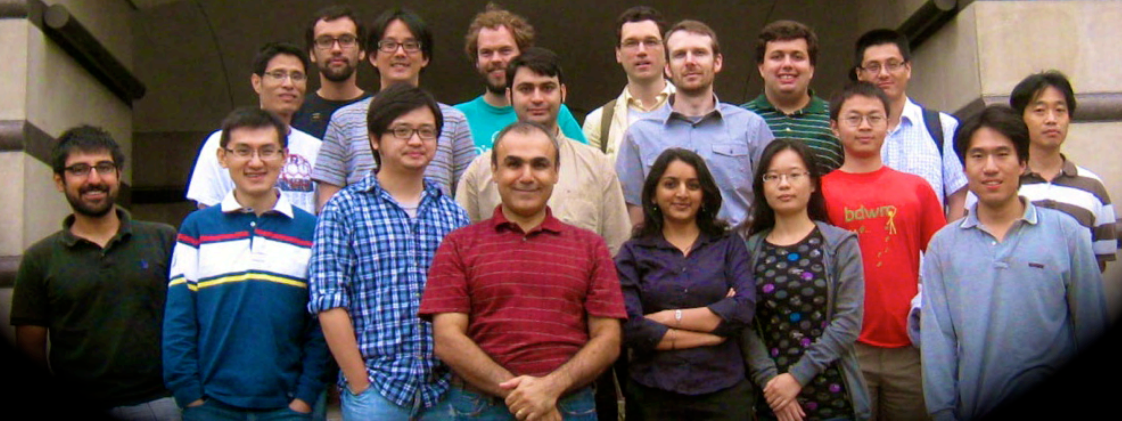
Computational Structural Biology and Molecular Biophysics Group (CSBMB)



Saher Shaikh



Yi Wang



csbmb.beckman.illinois.edu



TeraGrid™



Anton

Collaborators:

- Walter Boron
- Raif Musa-Aziz
- Xue Qin
- Robert Gennis

Transporter Team

Zhijian Huang

Jing Li

Giray Enkavi

Mahmoud Moradi

Wei Han

Saher Shaikh

Paween Mahin

Po-Chao Wen

Wenxun Gan

HMMM Team

Zenmei Ohkubo

Mark Arcario

Joshua Vermaas

Taras Pogorelov

Javier Baylon

R01-GM086749 U54-GM087519
R01-GM101048 P41-GM104601

Measuring Cellular CO₂ Permeability by ¹⁸O Exchange – Methodology and Results on Red Blood Cells

Gerolf Gros and Volker Endeward

Zentrum Physiologie
Medizinische Hochschule Hannover
Germany

Methods Available to Measure Membrane CO₂ Permeability

- Surface pH transients in *Xenopus* oocytes
- Kinetics of cellular CO₂ uptake recorded by intracellular pH measurement
- pH gradients in the surface region of epithelial cell layers
- Stopped flow rapid reaction spectrophotometry
- ¹⁸O exchange between CO₂, HCO₃⁻ and H₂O

Earlier Measurements of CO₂ permeability of membranes

P_{CO₂} of planar phospholipid bilayers from CO₂ flux measurements

0.35 cm/s (Gutknecht et al., 1977)

3.2 cm/s (Missner et al., 2008)

P_{CO₂} of phospholipid vesicles by stopped flow spectrophotometry

~ 10⁻³ cm/s (Prasad et al., 1998)

~ 10⁻³ cm/s (Yang et al., 2000)

Can the kinetics of CO₂ and O₂ uptake by red cells be reliably measured by stopped flow techniques?

$t_{1/2}$ of CO₂ uptake by human red cells: 13 ms

(Holland and Forster, 1975)

continuous-flow rapid reaction apparatus

$t_{1/2}$ of CO₂ uptake by red cells by theory: ~ 12 ms

(Endeward et al., 2008)

$t_{1/2}$ of O₂ uptake by human red cells: ~ 80 ms

(Vandegriff and Olson, 1984)

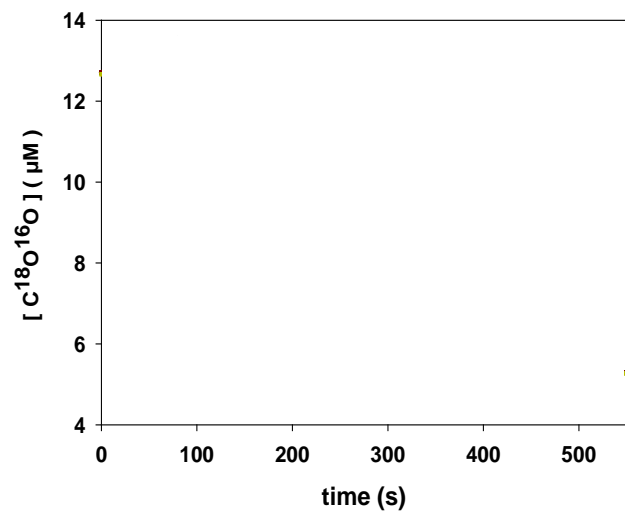
Determining Membrane Permeabilities of CO_2 and HCO_3^- by the ^{18}O Exchange Technique

Has been applied to

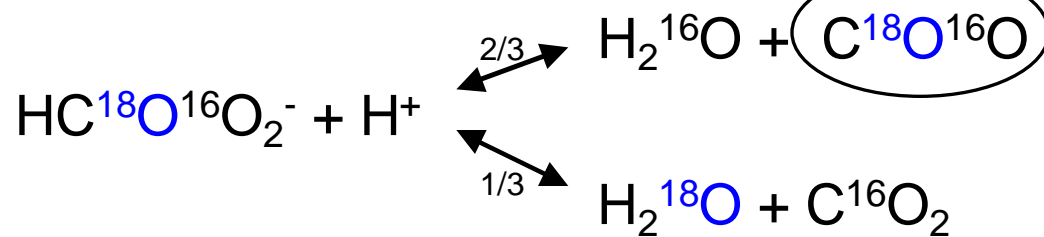
Isolated cells in suspension: red blood cells, MDCK and tsA201 cells

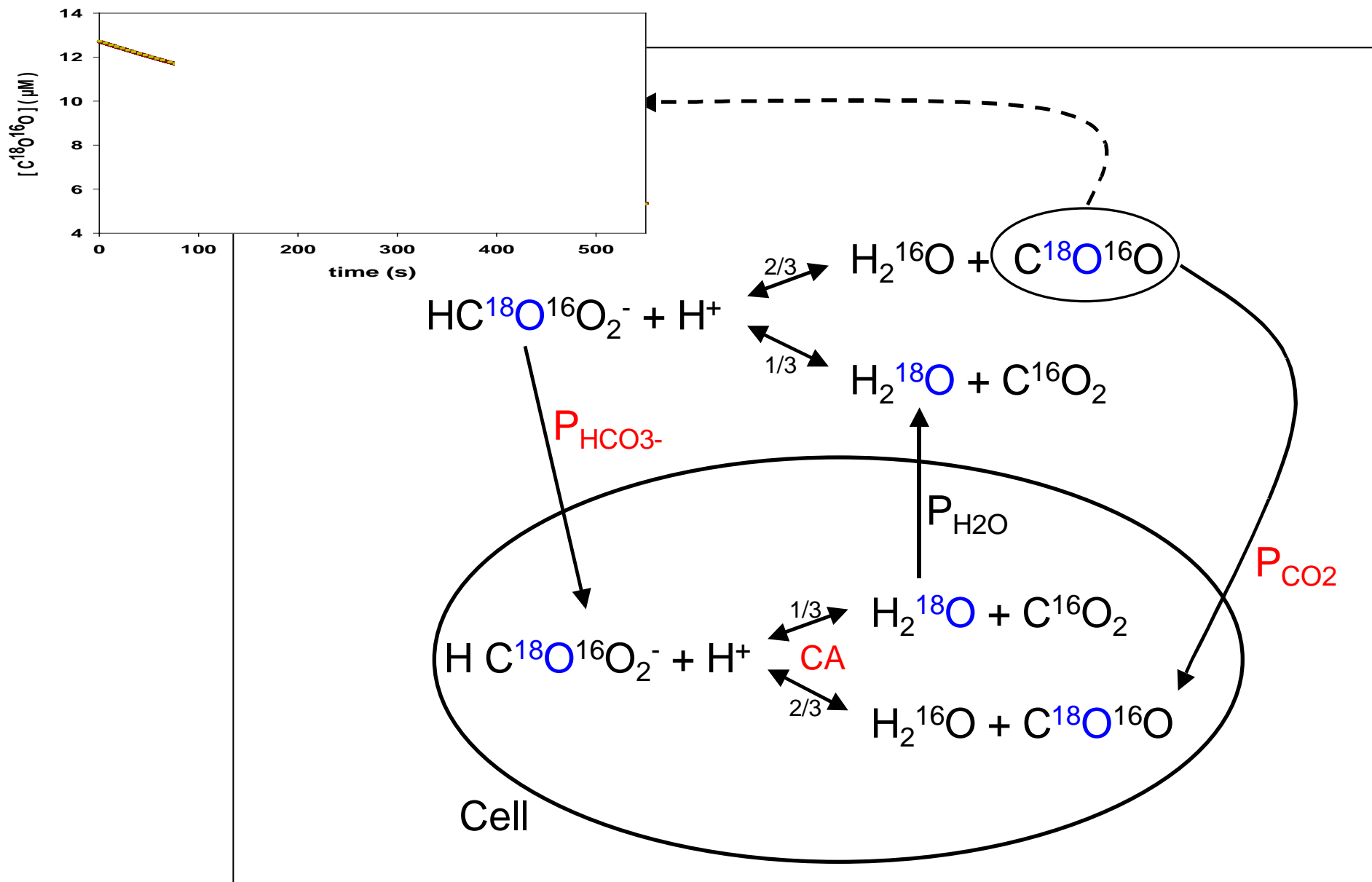
Phospholipid vesicles in suspension

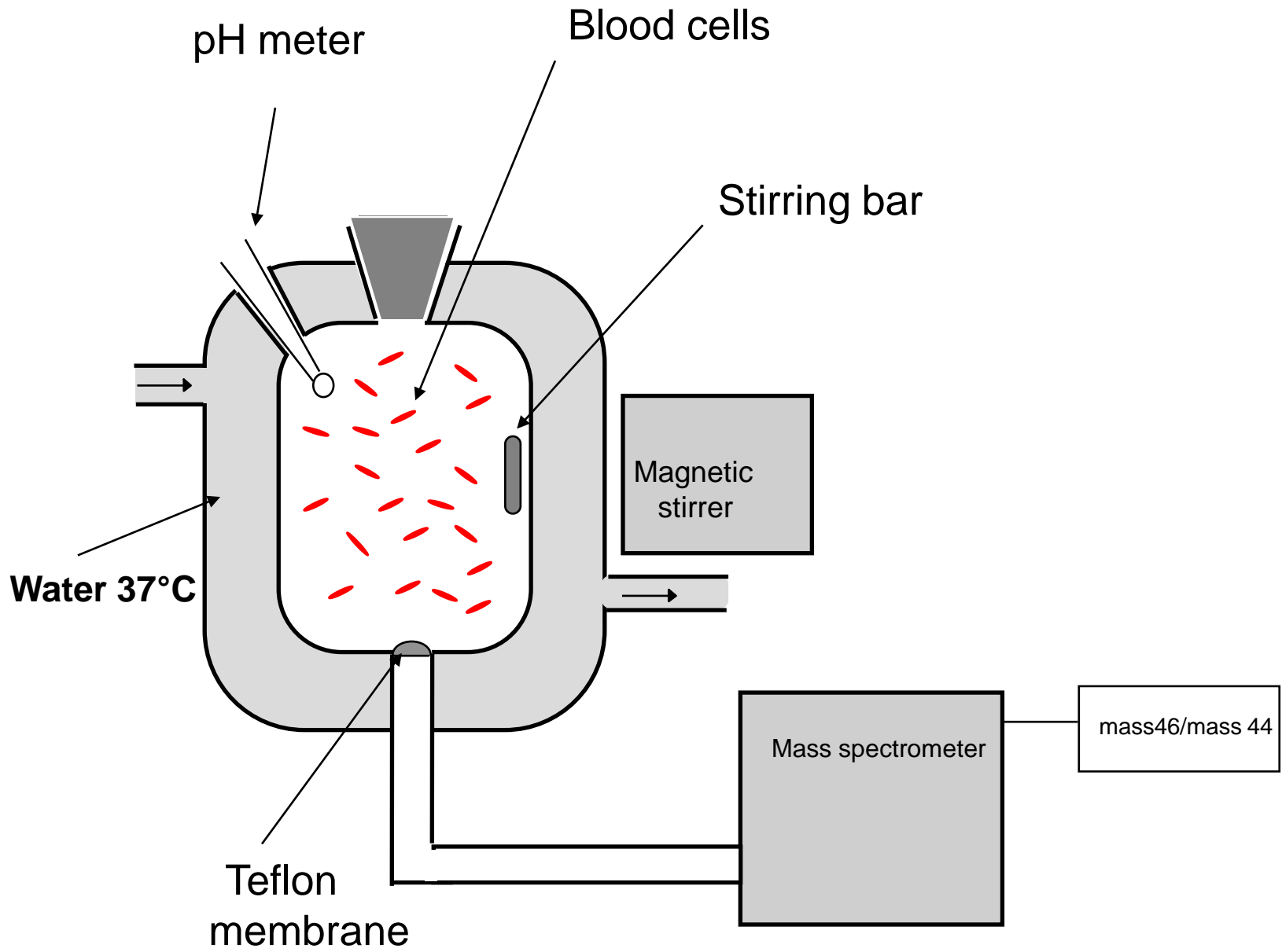
Intact colon epithelium

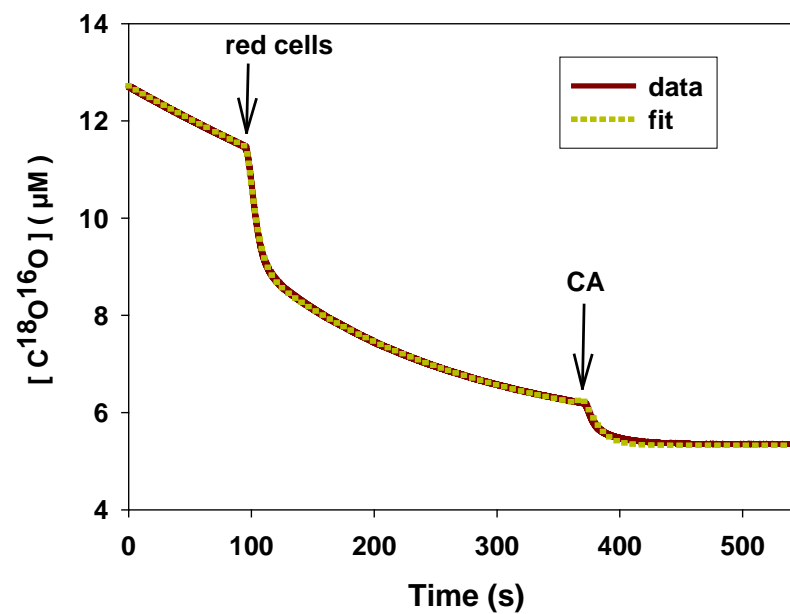
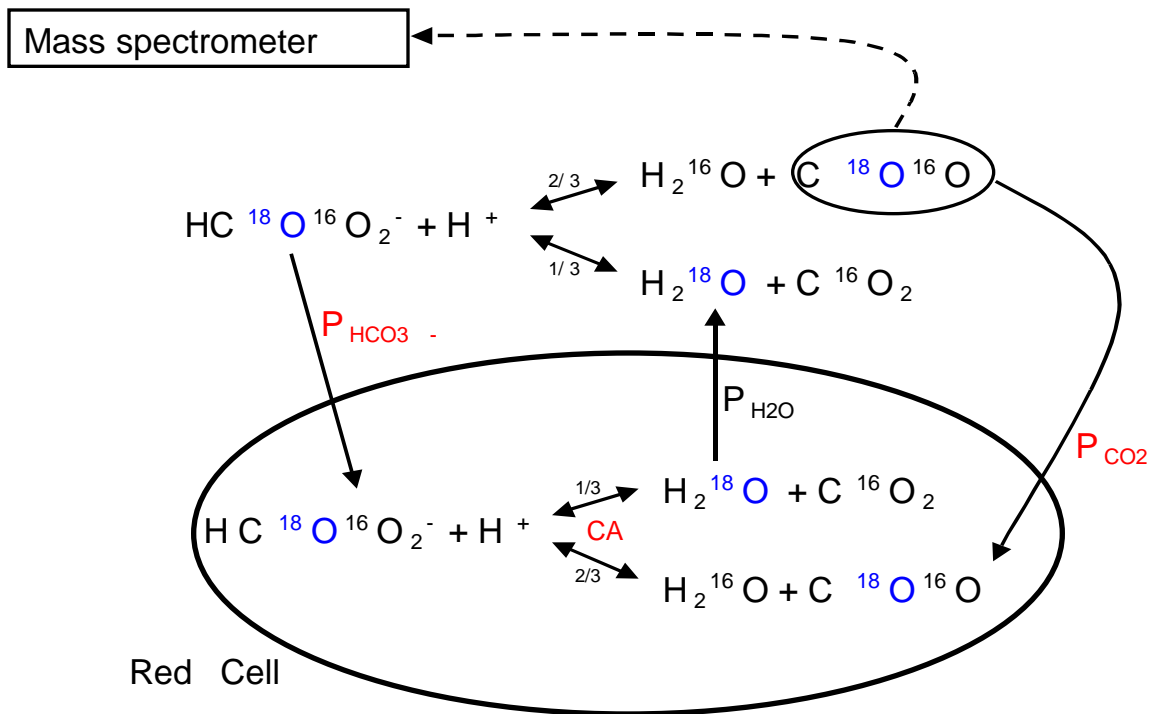


Mass spectrometer









$$\frac{d[\text{C}^{18}\text{O}^{16}\text{O}]_{\text{ex}}(t)}{dt} = -k_u A_{\text{ex}} [\text{C}^{18}\text{O}^{16}\text{O}]_{\text{ex}}(t) + \frac{2k_u}{3K_1'} A_{\text{ex}} [\text{H}^+]_{\text{ex}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{ex}}(t) + P_{\text{CO}_2} a \frac{v}{1-v} \{ [\text{C}^{18}\text{O}^{16}\text{O}]_{\text{in}}(t) - [\text{C}^{18}\text{O}^{16}\text{O}]_{\text{ex}}(t) \}$$

$$\frac{d[\text{C}^{18}\text{O}^{16}\text{O}]_{\text{in}}(t)}{dt} = -k_u A_{\text{in}} [\text{C}^{18}\text{O}^{16}\text{O}]_{\text{in}}(t) + \frac{2k_u}{3K_1'} A_{\text{in}} [\text{H}^+]_{\text{in}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{in}}(t) - P_{\text{CO}_2} a \{ [\text{C}^{18}\text{O}^{16}\text{O}]_{\text{in}}(t) - [\text{C}^{18}\text{O}^{16}\text{O}]_{\text{ex}}(t) \}$$

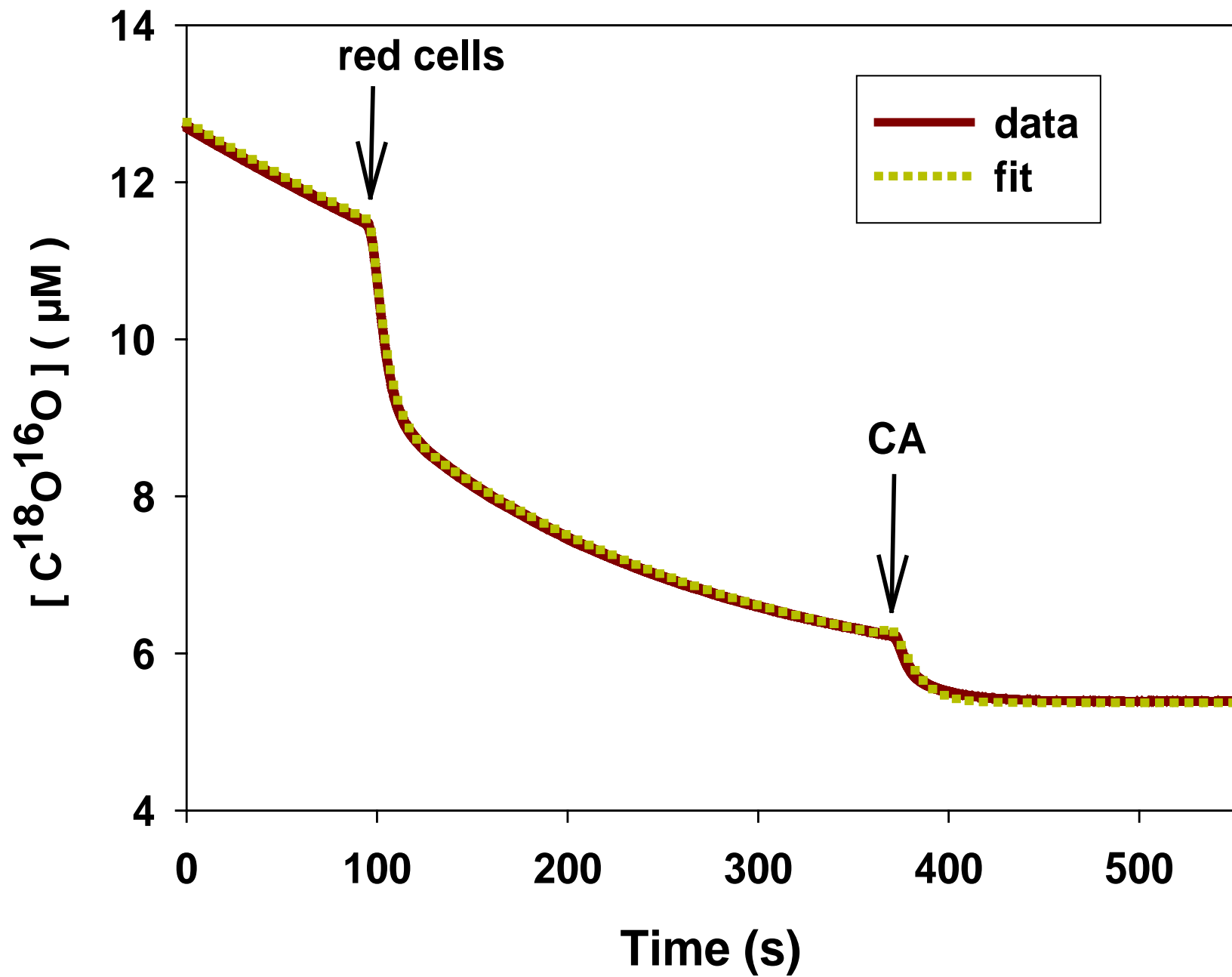
$$\frac{d[\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{ex}}(t)}{dt} = k_u A_{\text{ex}} \left([\text{C}^{18}\text{O}^{16}\text{O}]_{\text{ex}}(t) + [\text{CO}_2] \frac{[\text{H}_2^{18}\text{O}]_{\text{ex}}(t)}{[\text{H}_2\text{O}]} \right) - \frac{k_u}{K_1'} A_{\text{ex}} [\text{H}^+]_{\text{ex}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{ex}}(t) - P_{\text{HCO}_3^-} a \frac{v}{1-v} \left\{ \frac{[\text{H}^+]_{\text{ex}}}{[\text{H}^+]_{\text{in}}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{ex}}(t) - [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{in}}(t) \right\}$$

$$\frac{d[\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{in}}(t)}{dt} = k_u A_{\text{in}} \left([\text{C}^{18}\text{O}^{16}\text{O}]_{\text{in}}(t) + [\text{CO}_2] \frac{[\text{H}_2^{18}\text{O}]_{\text{in}}(t)}{[\text{H}_2\text{O}]} \right) - \frac{k_u}{K_1'} A_{\text{in}} [\text{H}^+]_{\text{in}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{in}}(t) - P_{\text{HCO}_3^-} a \left\{ \frac{[\text{H}^+]_{\text{ex}}}{[\text{H}^+]_{\text{in}}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{ex}}(t) - [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{in}}(t) \right\}$$

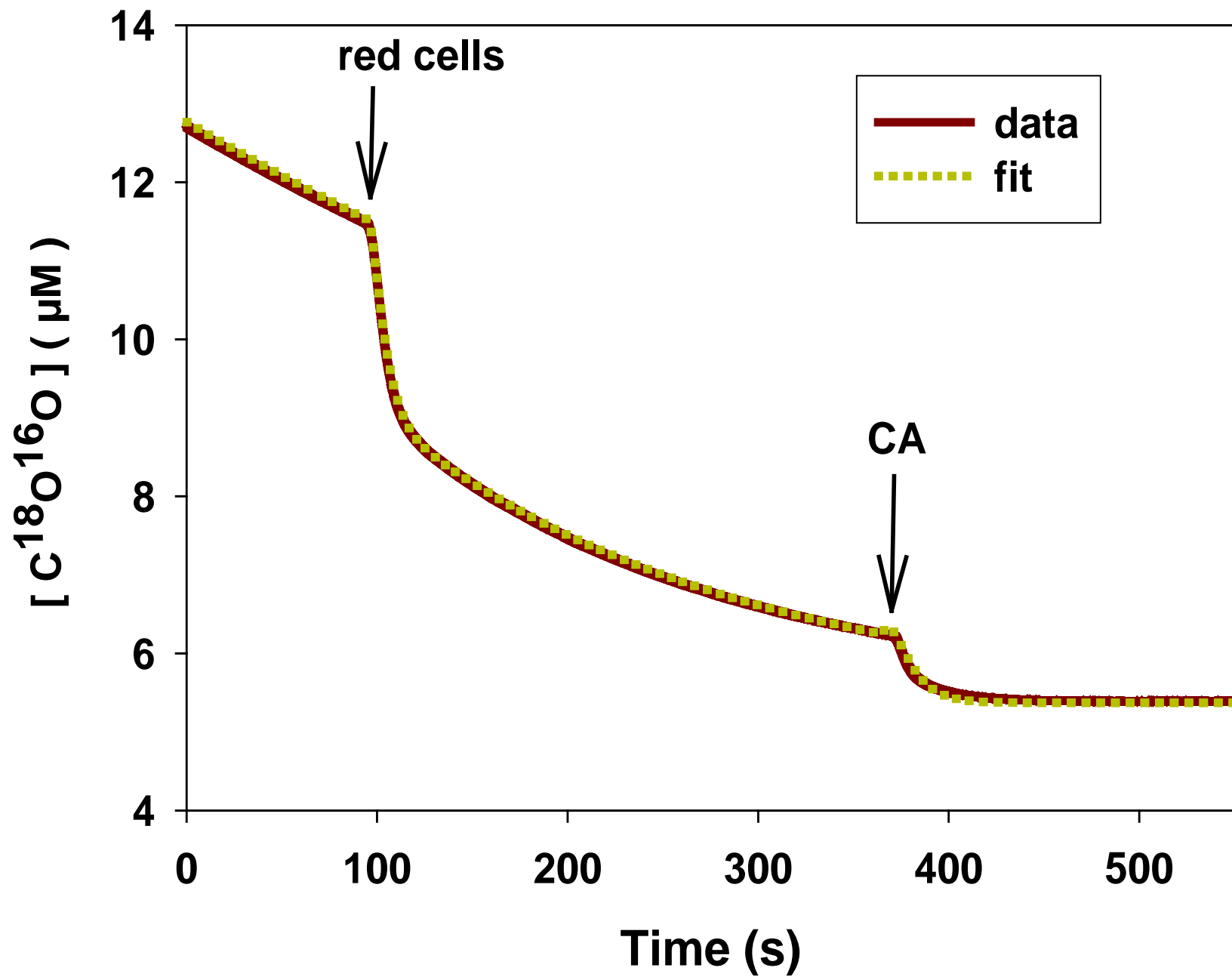
$$\frac{d[\text{H}_2^{18}\text{O}]_{\text{ex}}(t)}{dt} = \frac{1k_u}{3K_1'} A_{\text{ex}} [\text{H}^+]_{\text{ex}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{ex}}(t) - k_u A_{\text{ex}} \frac{[\text{CO}_2]}{[\text{H}_2\text{O}]} [\text{H}_2^{18}\text{O}]_{\text{ex}}(t) + P_{\text{H}_2\text{O}} a \frac{v}{1-v} \{ [\text{H}_2^{18}\text{O}]_{\text{in}}(t) - [\text{H}_2^{18}\text{O}]_{\text{ex}}(t) \}$$

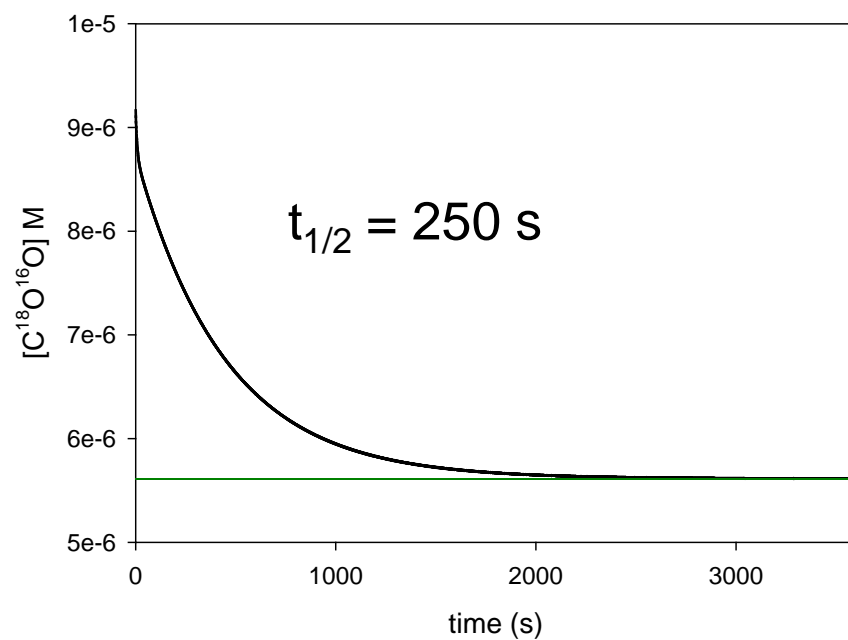
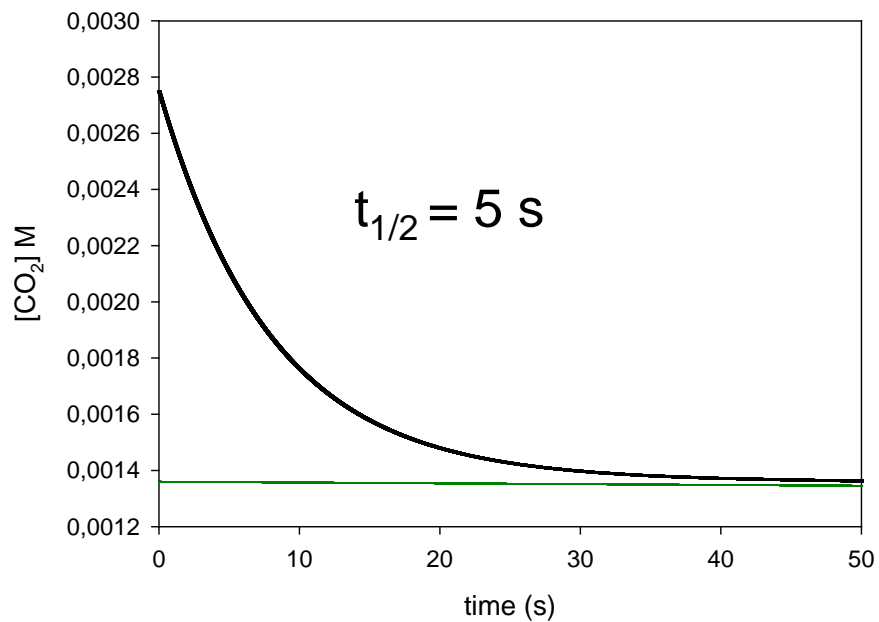
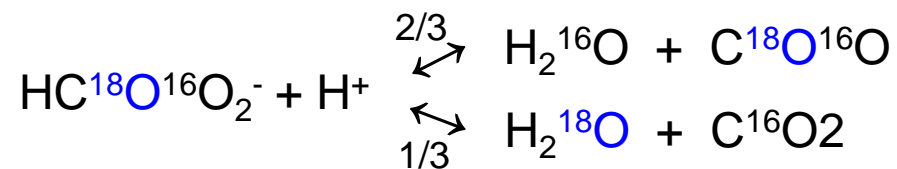
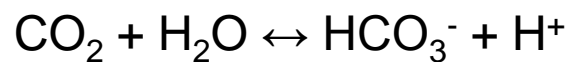
$$\frac{d[\text{H}_2^{18}\text{O}]_{\text{in}}(t)}{dt} = \frac{1k_u}{3K_1'} A_{\text{in}} [\text{H}^+]_{\text{in}} [\text{HC}^{18}\text{O}^{16}\text{O}_2^-]_{\text{in}}(t) - k_u A_{\text{in}} \frac{[\text{CO}_2]}{[\text{H}_2\text{O}]} [\text{H}_2^{18}\text{O}]_{\text{in}}(t) - P_{\text{H}_2\text{O}} a \{ [\text{H}_2^{18}\text{O}]_{\text{in}}(t) - [\text{H}_2^{18}\text{O}]_{\text{ex}}(t) \}$$

Fig.3

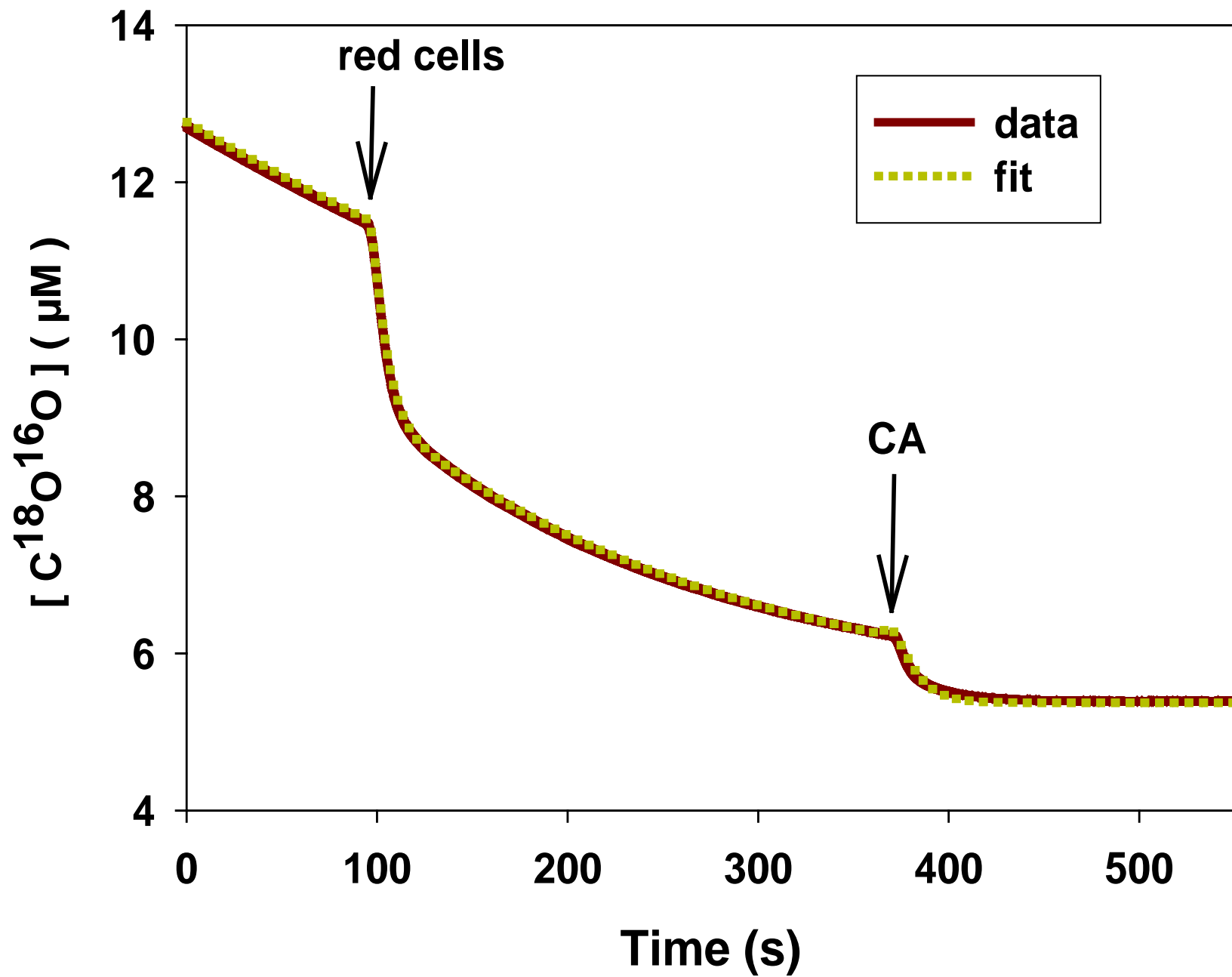


Why can we observe fast processes on such a slow time scale,
allowing us to follow these processes by mass spectrometry?





Kinetics of CO_2 hydration reaction vs. that of ^{18}O exchange



It was shown here that a time course of the decay of $[C^{18}O^{16}O]$ that is measurable by mass spectrometry, is observed when the volume fraction of human red cells is extremely small, i.e. 2×10^{-4} . Raising this volume fraction by a factor of 10, to 0.002, renders the signal already too fast compared to the time resolution of the mass spectrometer in combination with the inlet system.

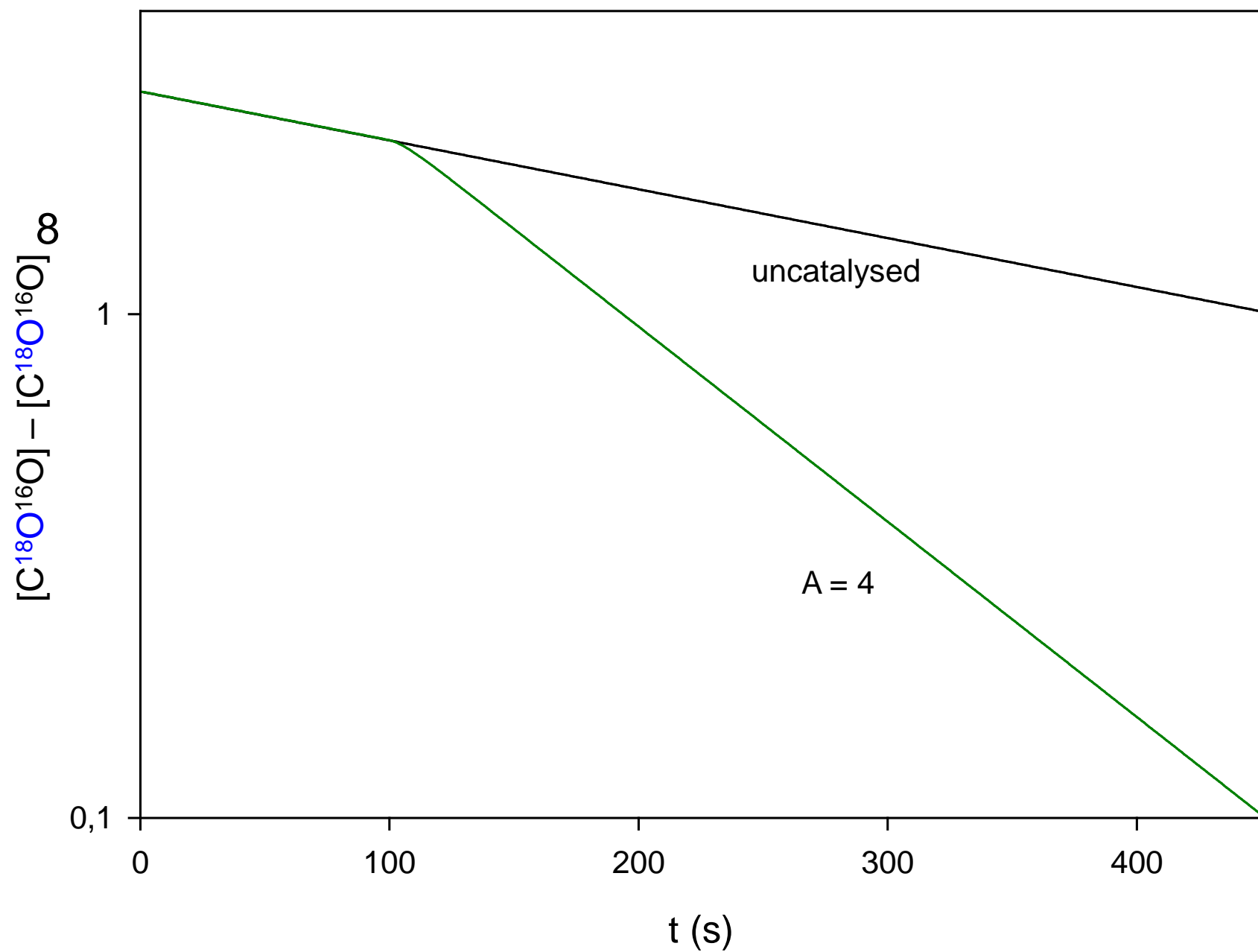
It is concluded that the process of ^{18}O exchange can be slowed down by orders of magnitude, because it is possible to use extremely small amounts of red cells and still obtain a well-defined and clear signal.

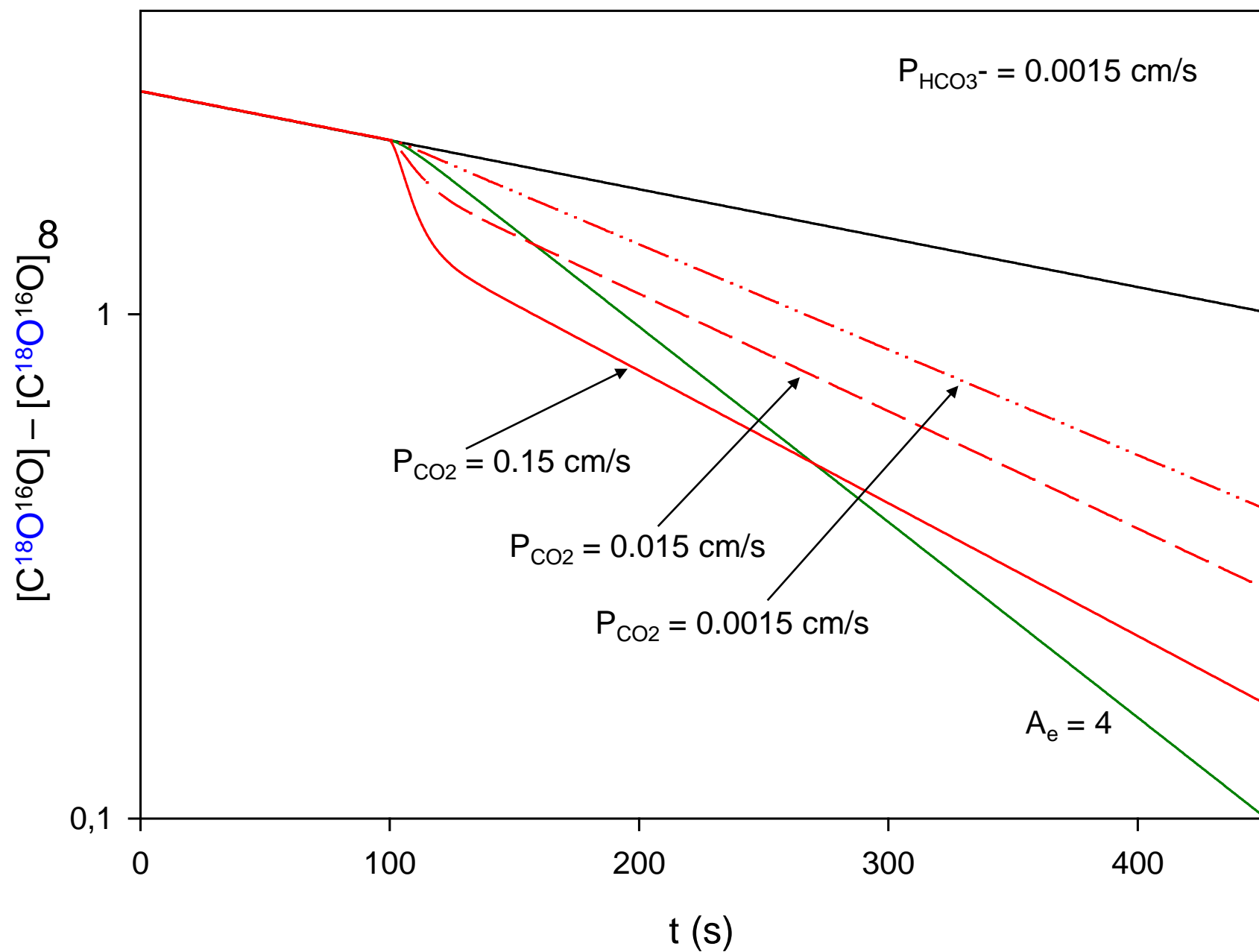
Also for this reason, the ^{18}O exchange technique allows us to observe fast processes such as the uptake of CO_2 by red cells on a very slow time scale.

How well are P_{CO_2} and $P_{\text{HCO}_3^-}$ defined by the experimental curves of decay of $[\text{C}^{18}\text{O}^{16}\text{O}]$?

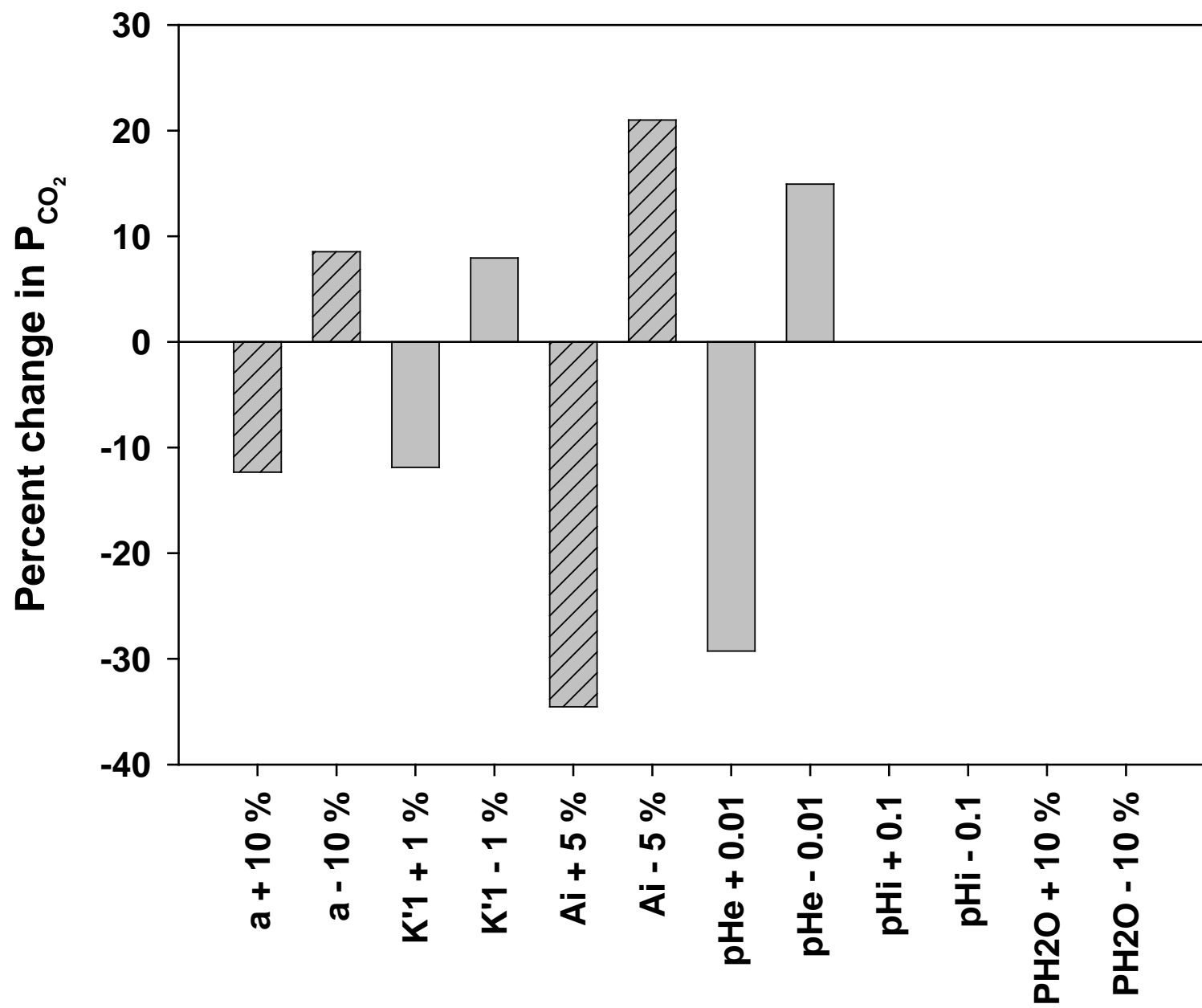
It was shown here that a well-defined minimum exists for both $P_{\text{HCO}_3^-}$ and P_{CO_2} in the sum of squares of deviations between the experimental data of $[\text{C}^{18}\text{O}^{16}\text{O}]$ and those obtained from the best-fit calculation.

When $P_{\text{HCO}_3^-}$ and P_{CO_2} are varied over a wide range of values, clearly only one well-defined minimum is apparent and no local minima whatsoever are visible.





Sensitivity of calculated P_{CO_2} to parameter values



To what extent do unstirred layers around cells
affect the permeability determinations?

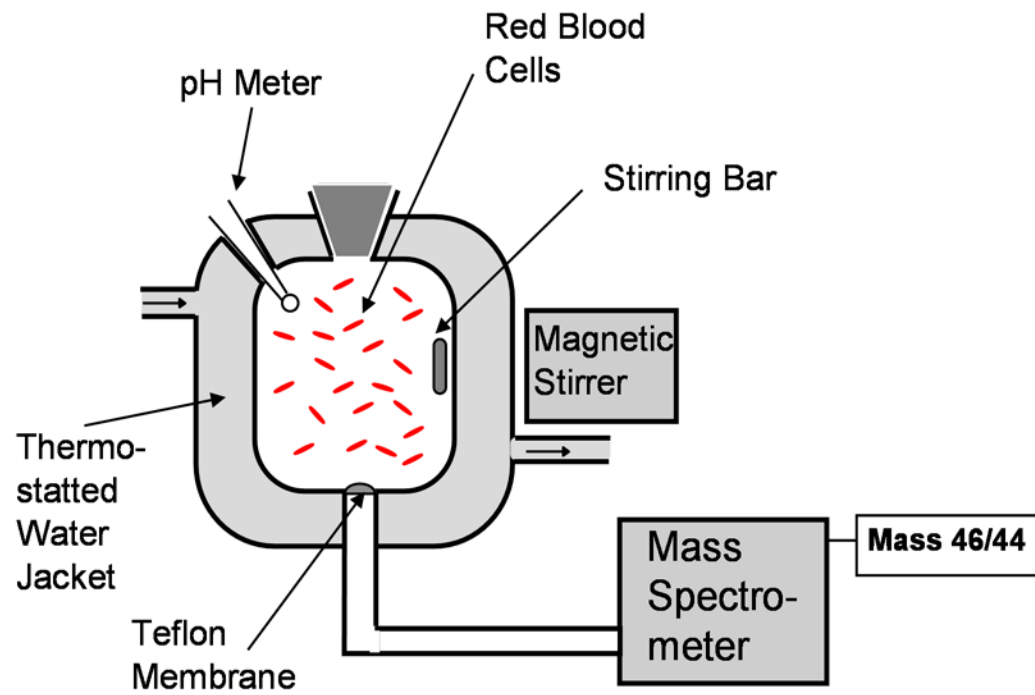
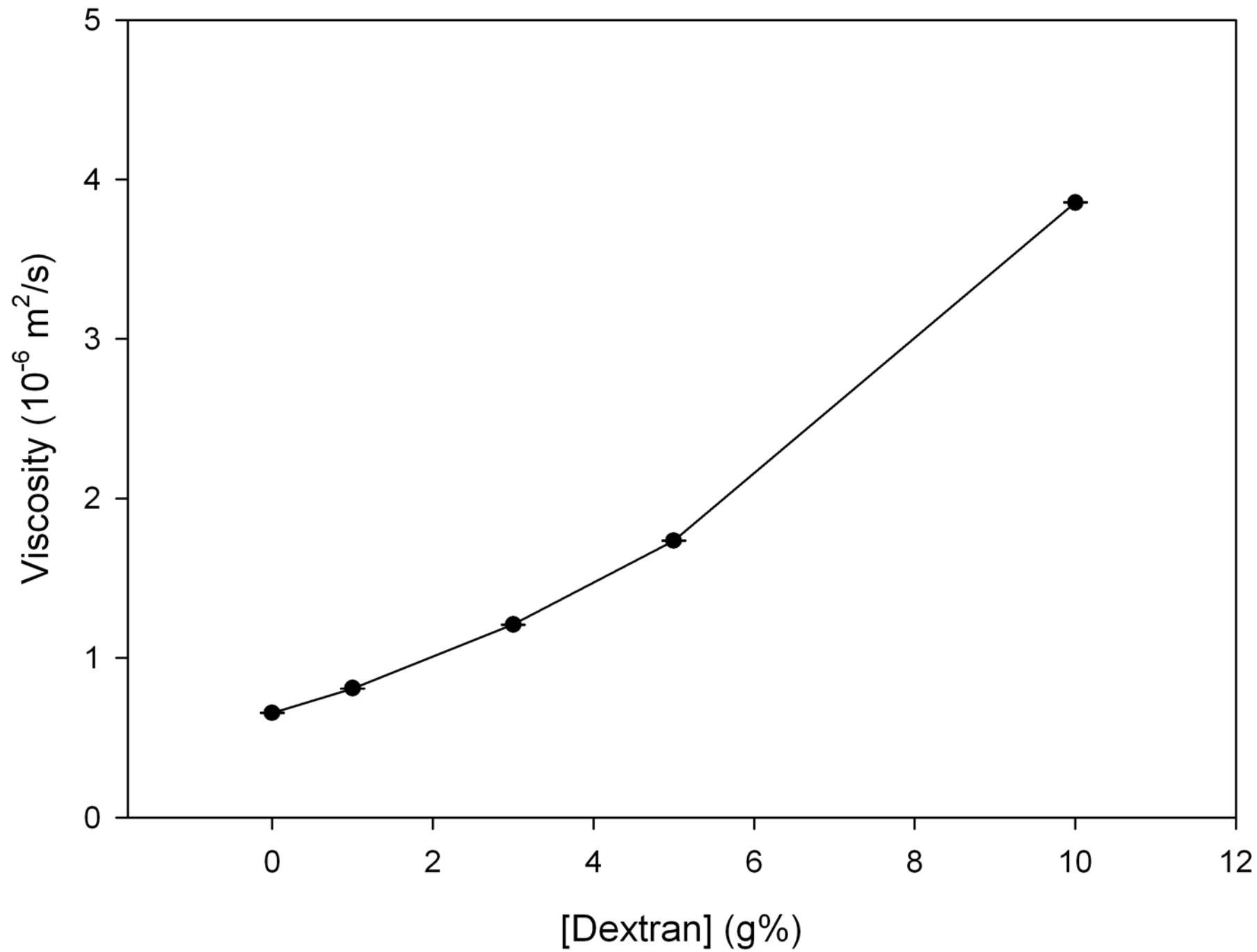


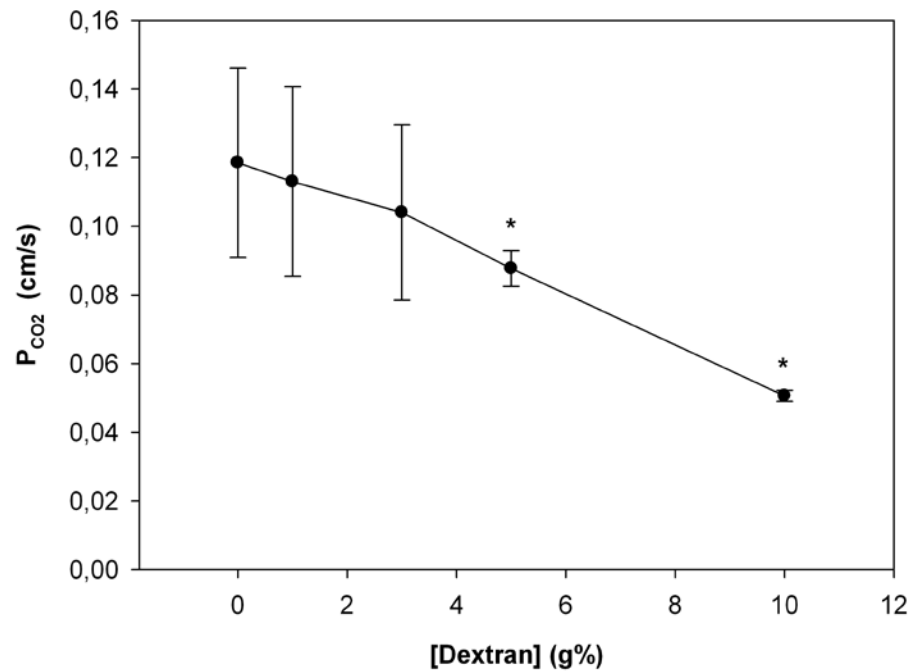
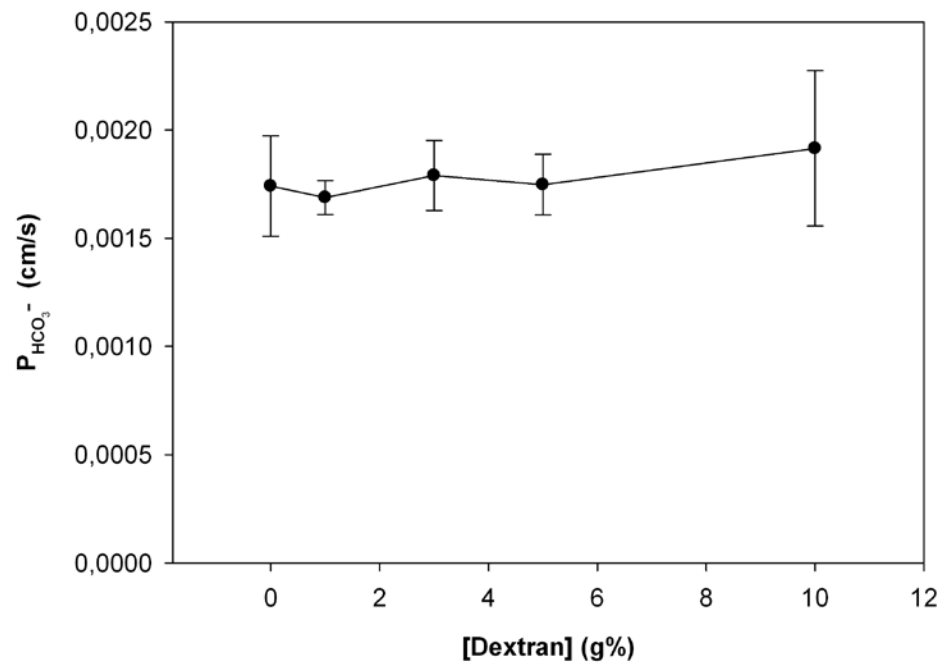
Fig. 1

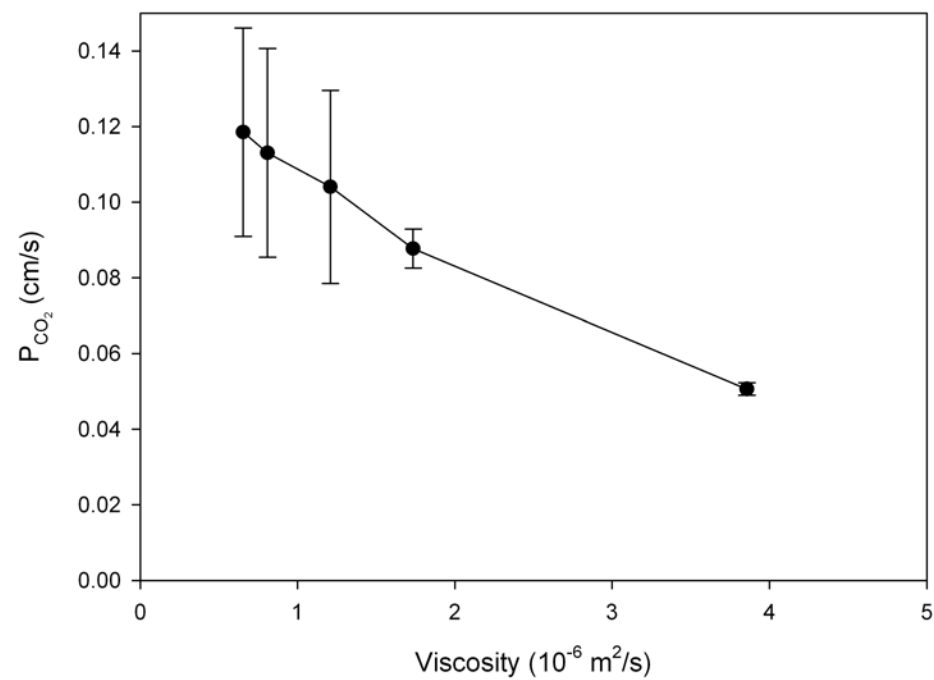
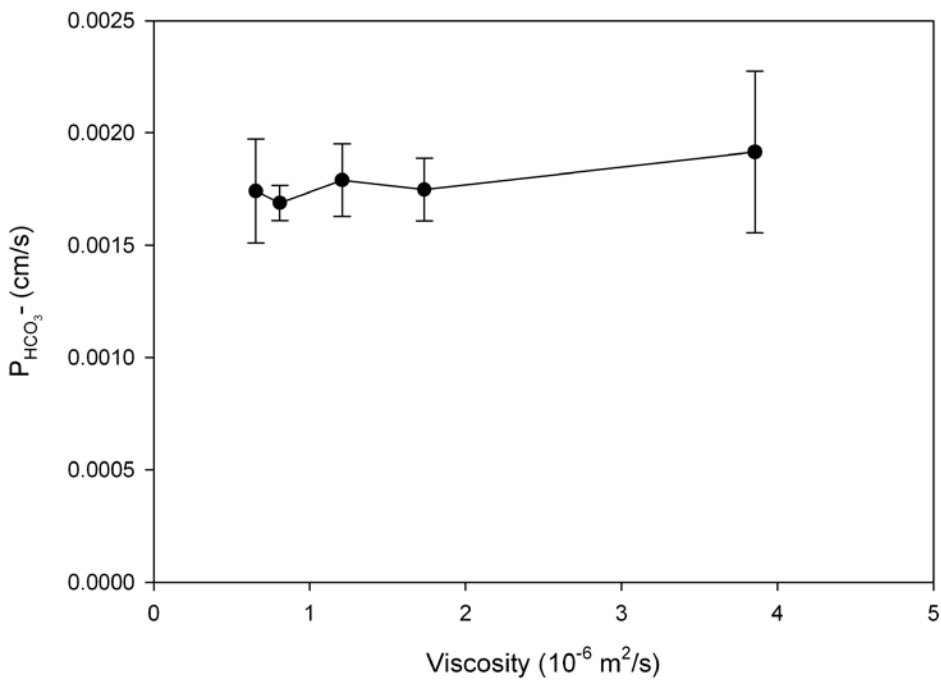
thickness of unstirred layer $\delta \sim$

kinematic viscosity $\nu \times \sqrt{\text{cell diameter } \ell}$

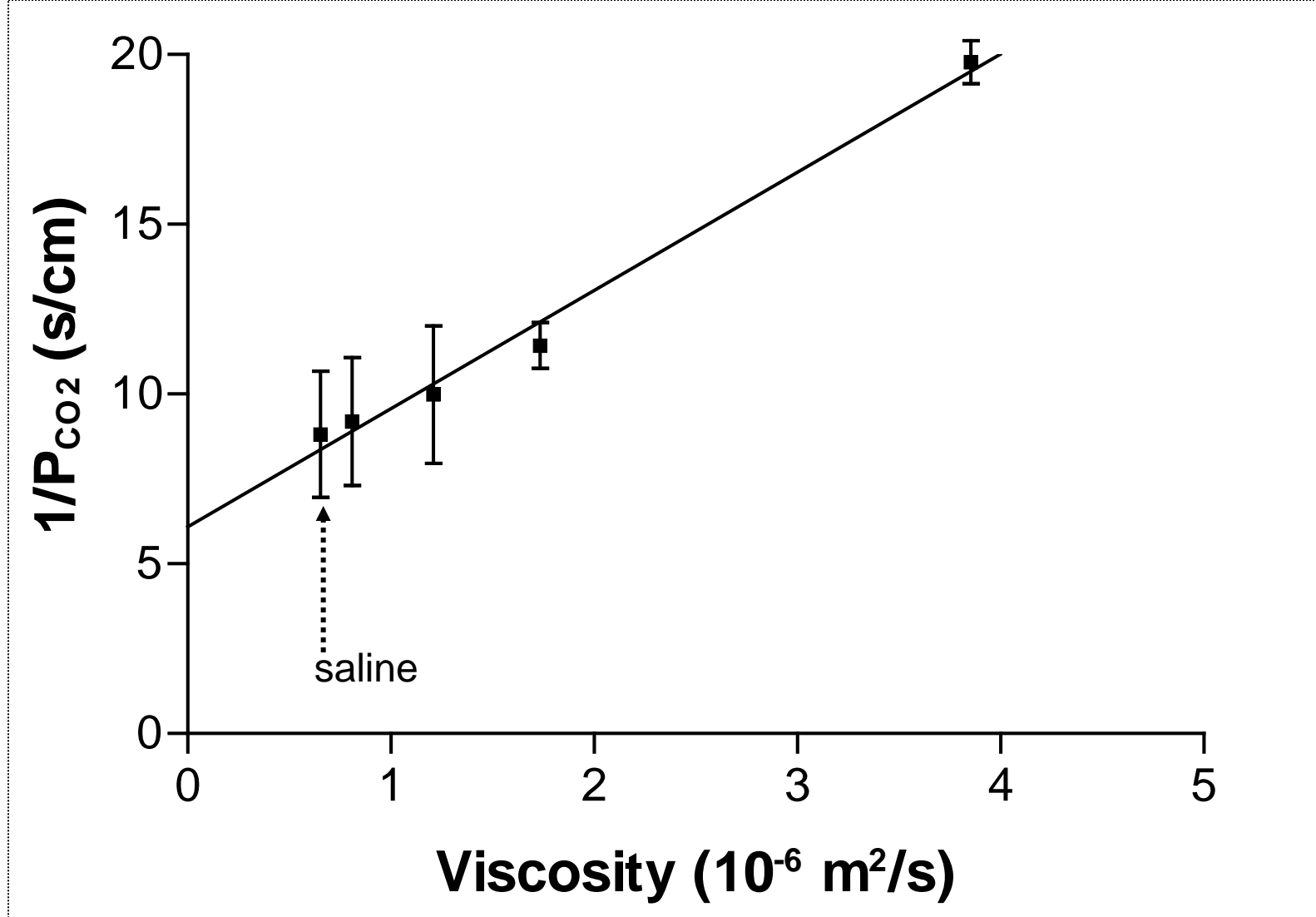
Landau LD and Lifschitz EM (1991): Hydrodynamics



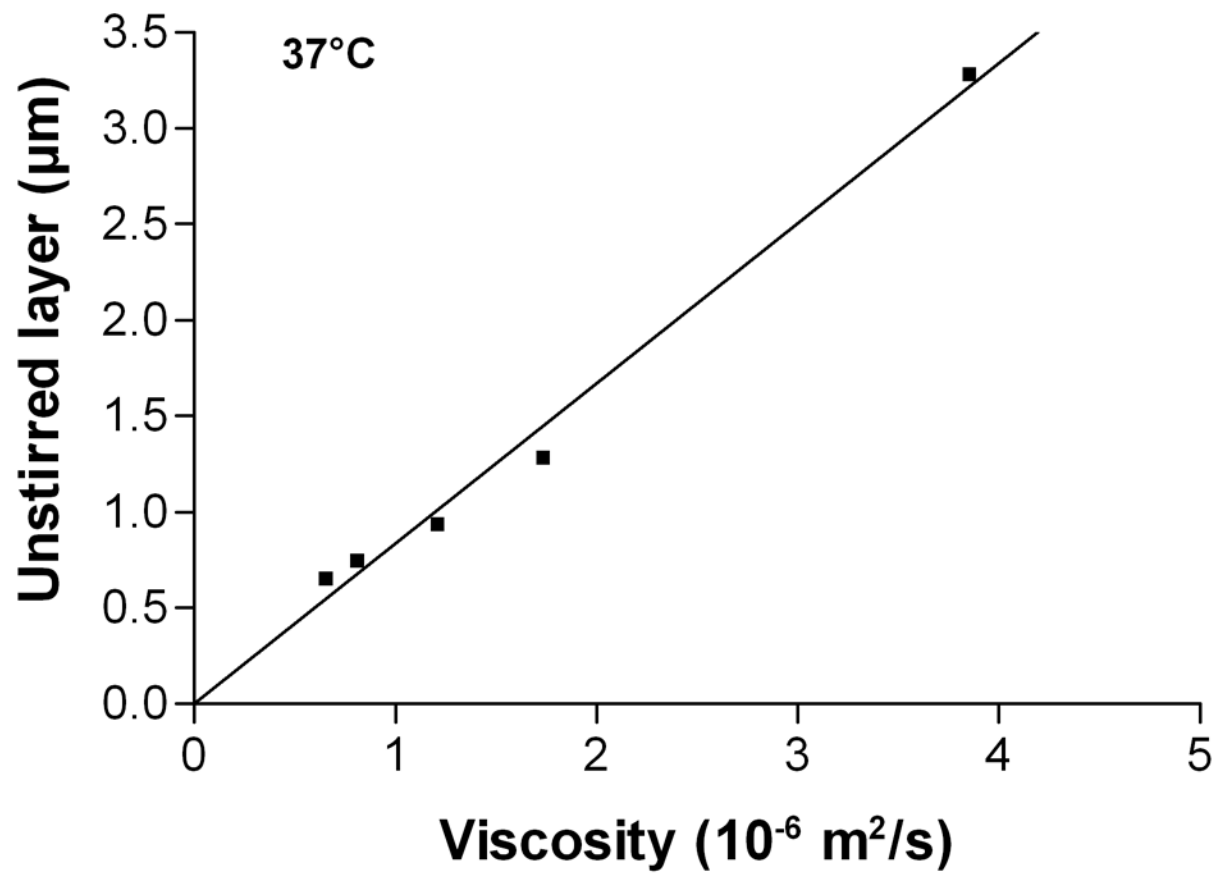




$$\frac{1}{P_{CO_2,app}} = \frac{1}{P_{CO_2,mem}} + \frac{\delta_{UL_e}}{D_{CO_2,solution}}$$



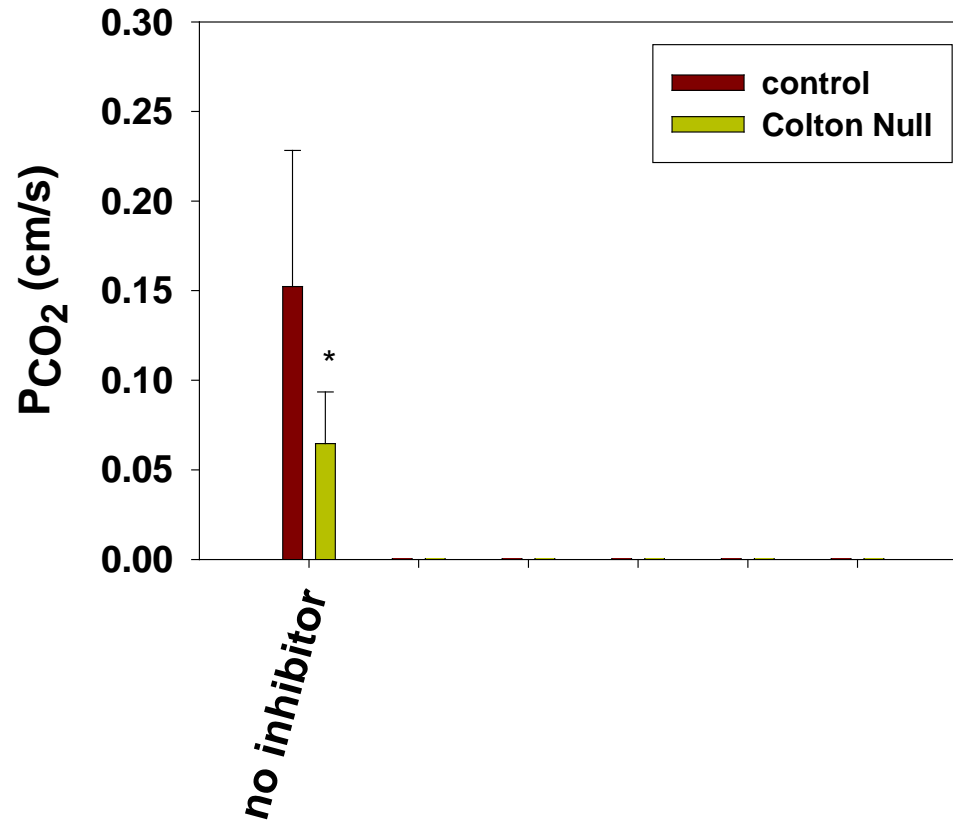
	P_{app} in saline (cm/s)	P_{M} (cm/s)	δ in saline (μm)
37 °C	0.12	0.16	0.5

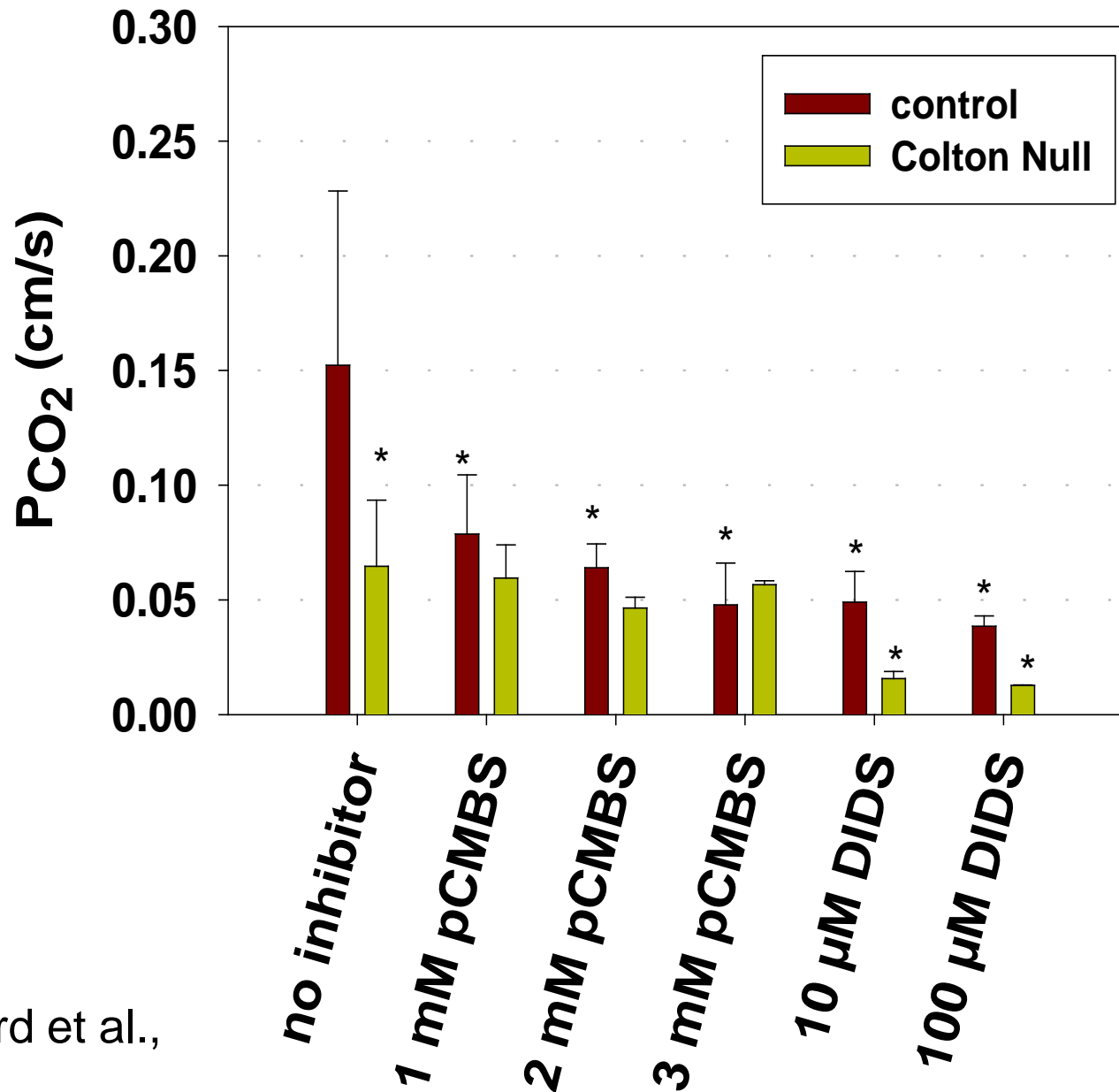


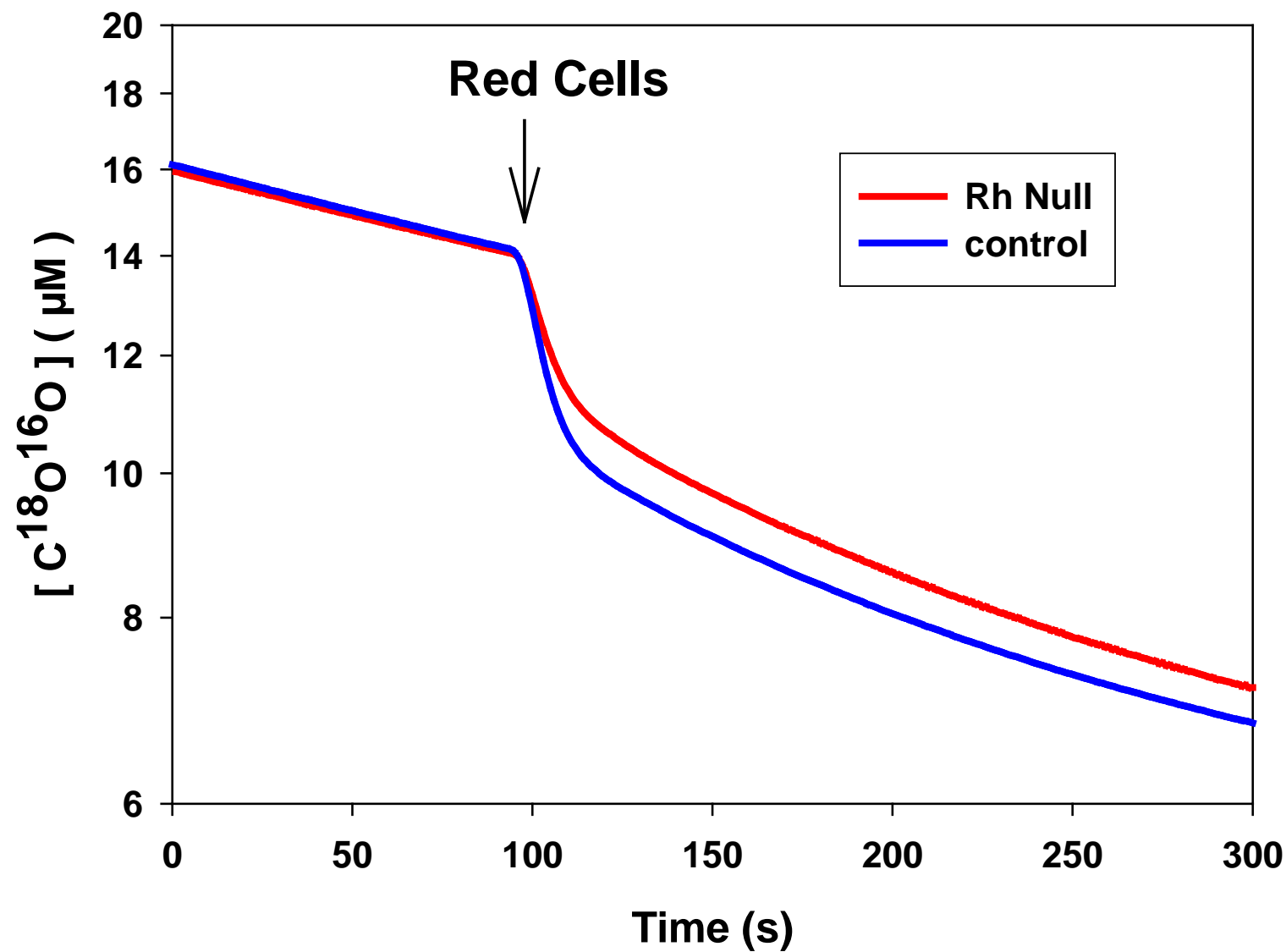
$$\delta \sim \nu$$

CO₂ Permeability of Normal and Deficient Human Red Blood Cells

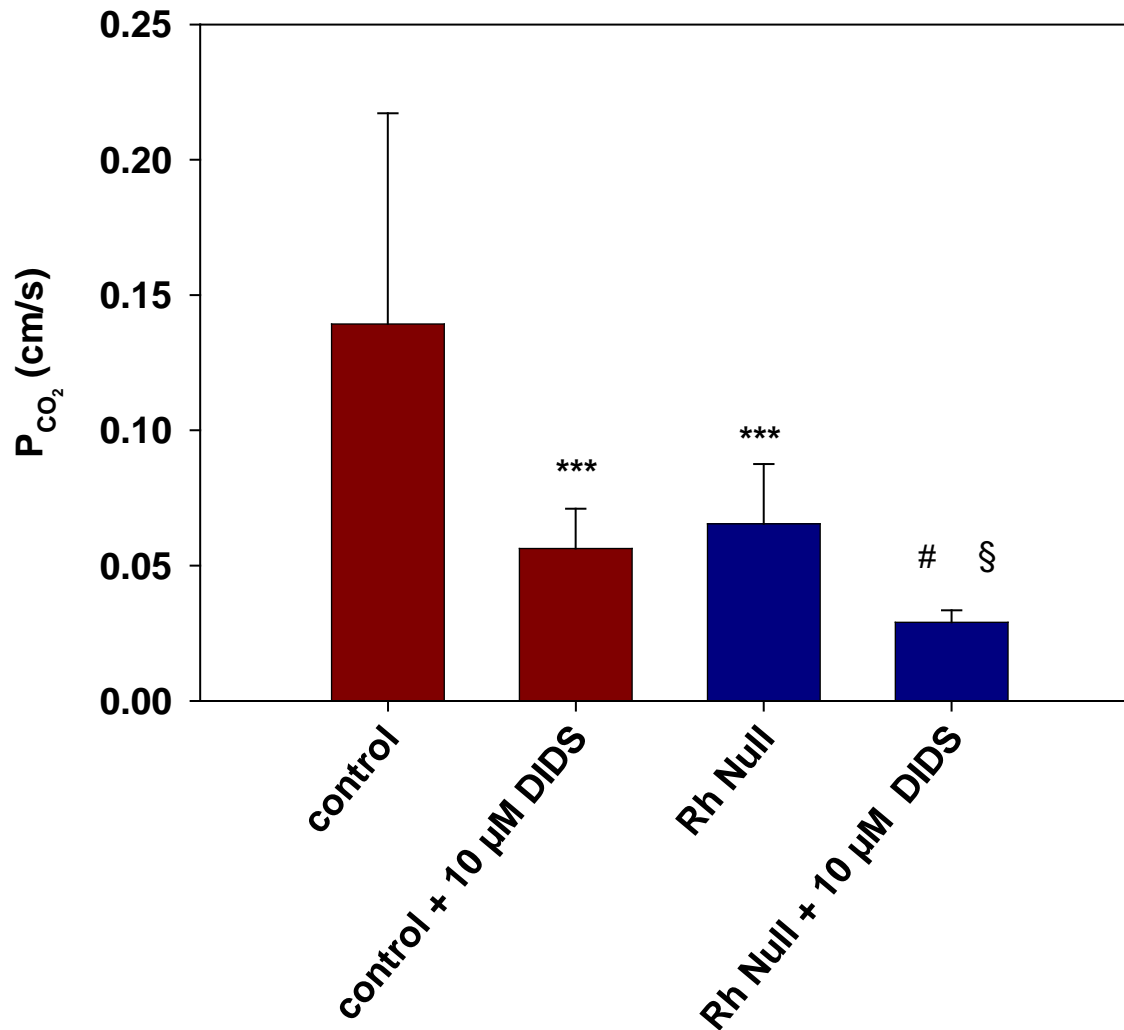
P_{CO_2} of control and AQP1 deficient (Colton null) human red blood cells





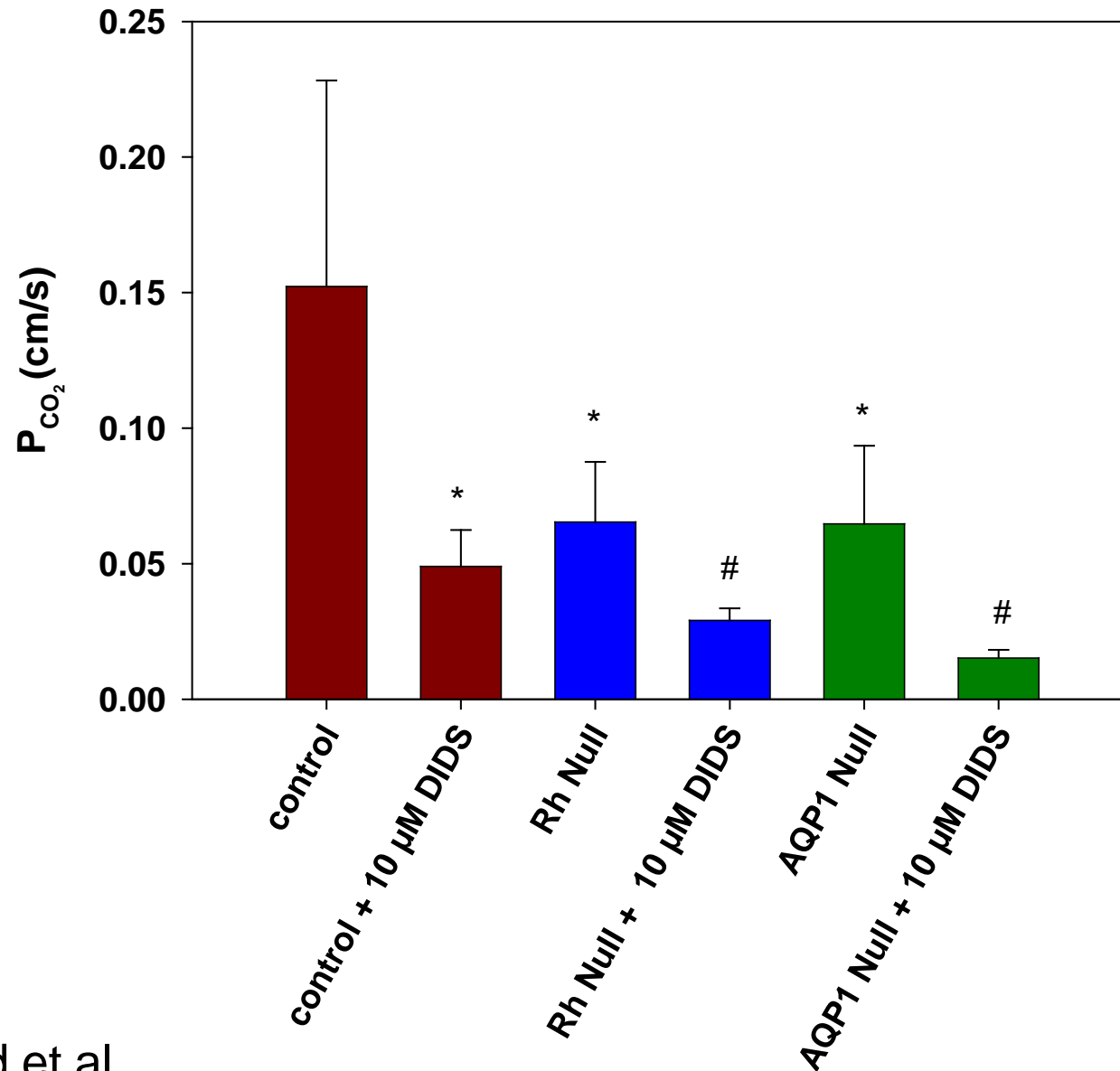


P_{CO_2} of control and Rhesus null human red blood cells



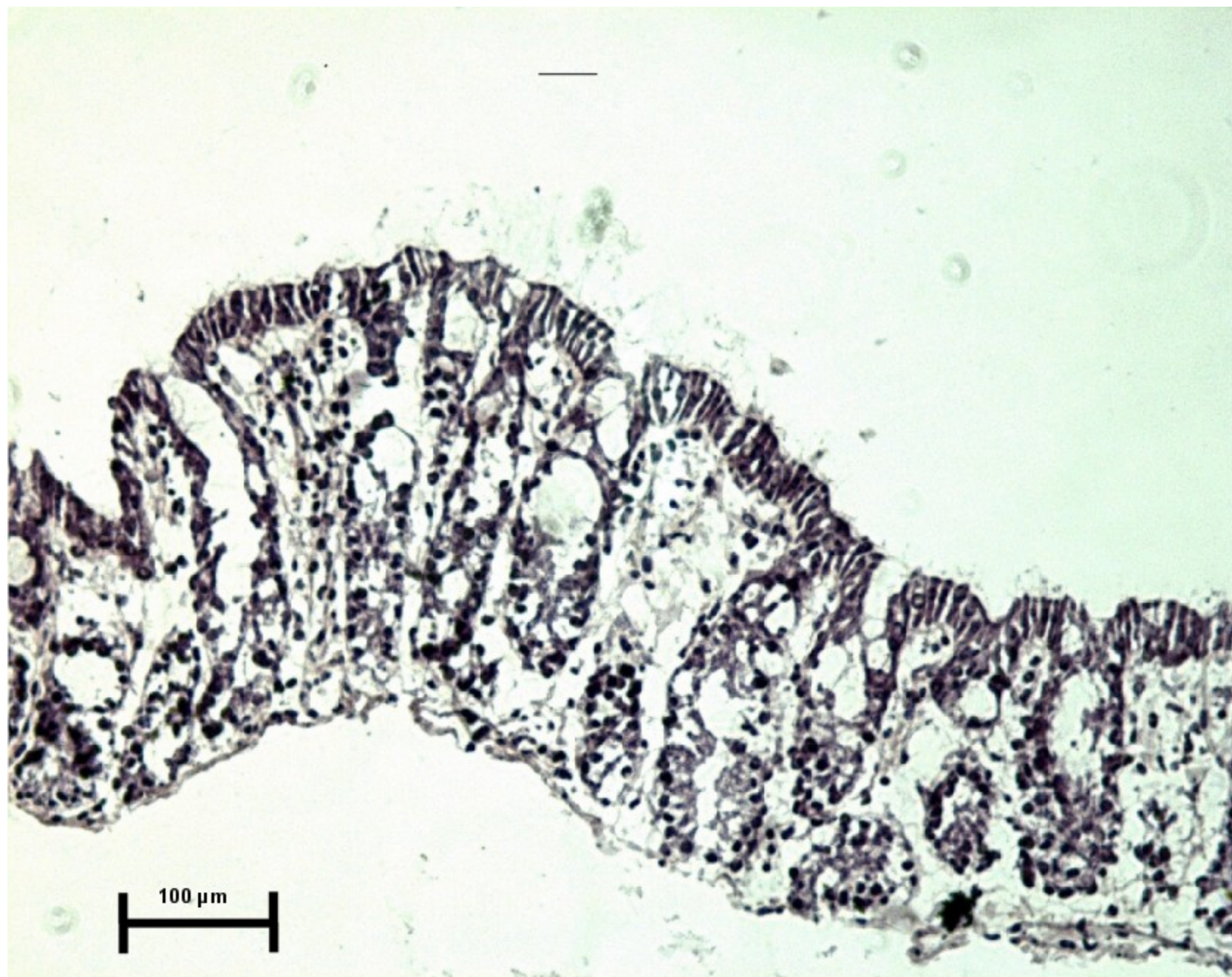
Endeward et al.,
FASEB J, 2008

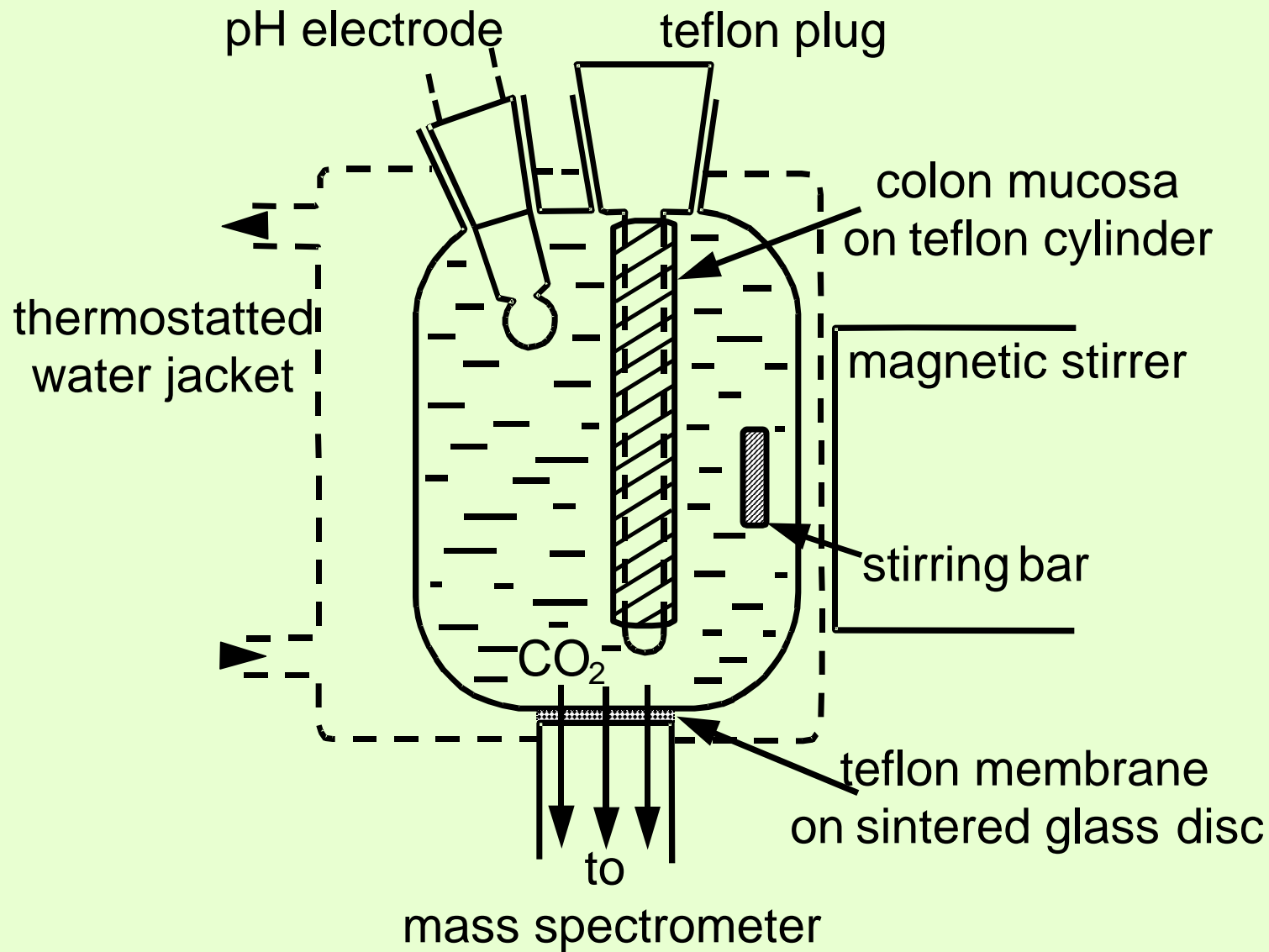
Human Red Blood Cell



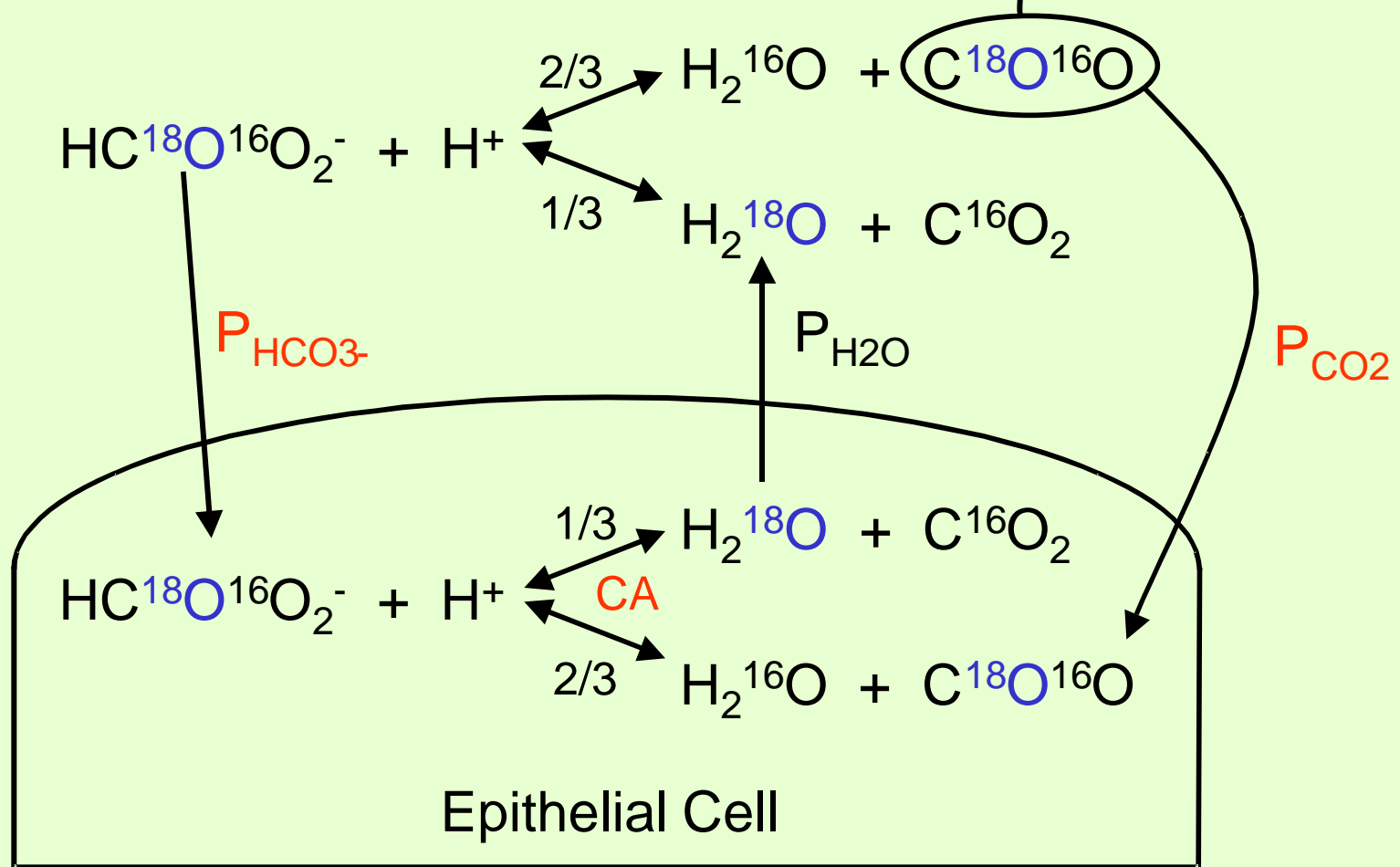
Endeward et al.,
2006, 2008

Applying the ^{18}O technique to measure the
 CO_2 permeability of the apical membrane of
intact colon epithelium

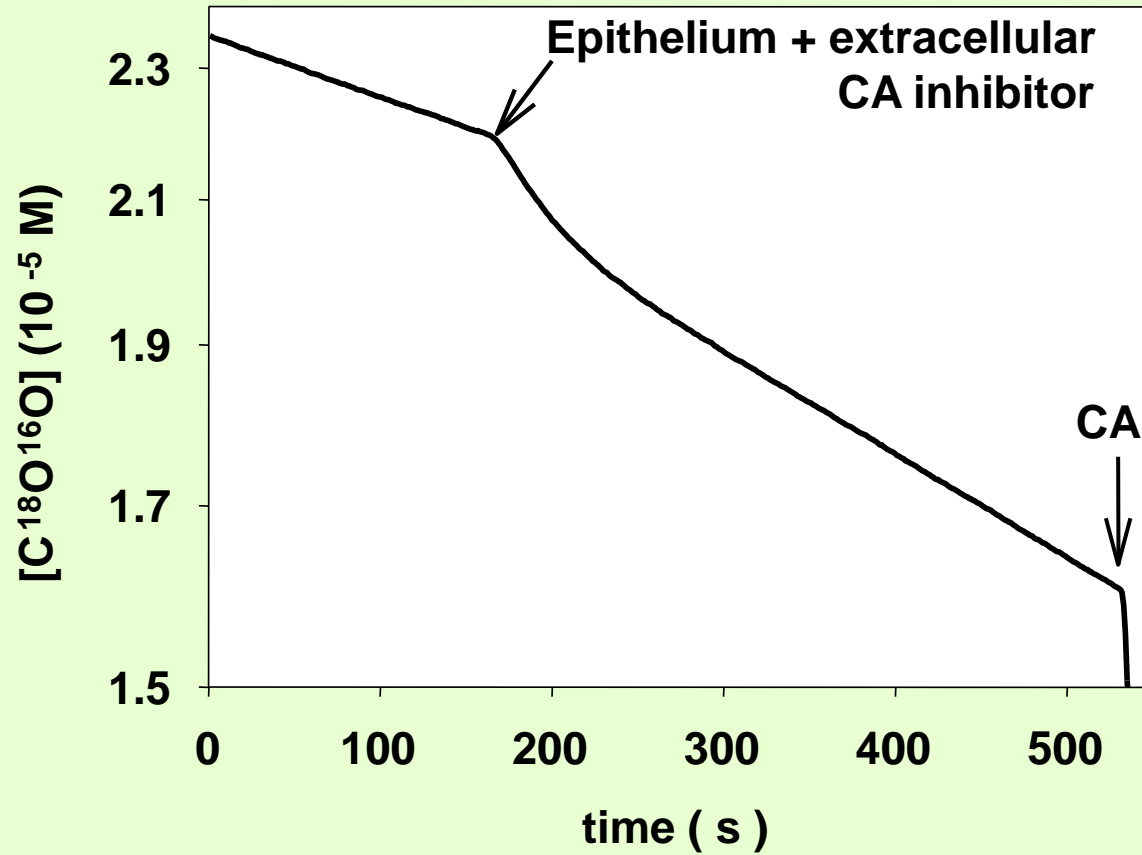




Mass Spectrometer



Intact Proximal Epithelium Apical Side



CO₂ and HCO₃⁻ Permeability of the Apical Membrane of Intact Guinea Pig Colon

	P_{CO2} ± SD (cm/s)	P_{HCO3⁻} (cm/s)	A_{in}	n
Intact Proximal Colon	0.0015 ± 0.0007	6.3 · 10⁻⁴ ± 4.0 · 10 ⁻⁴	41 000	40
Intact Distal Colon	0.00077 ± 0.00021	0.87 · 10⁻⁴ ± 0.56 · 10 ⁻⁴	900	23

Endeward & Gros, 2005

Conclusions

The ^{18}O exchange technique follows the decay of ^{18}O -labelled CO_2 in the extracellular fluid by mass spectrometry

This is possible because this decay is 1.000-10.000 times slower than net CO_2 uptake by cells or vesicles

The system of differential equations describing this process yields values of P_{CO_2} and $P_{\text{HCO}_3^-}$ from well defined minima of a fitting procedure

P_{CO_2} values can be determined over a range of 3-4 orders of magnitude

Parameters critical für calculation of P_{CO_2} and $P_{\text{HCO}_3^-}$ are intracellular CA activity and extracellular pH, both of which are carefully controlled

Unstirred layers affect the results by no more than ~ 20%

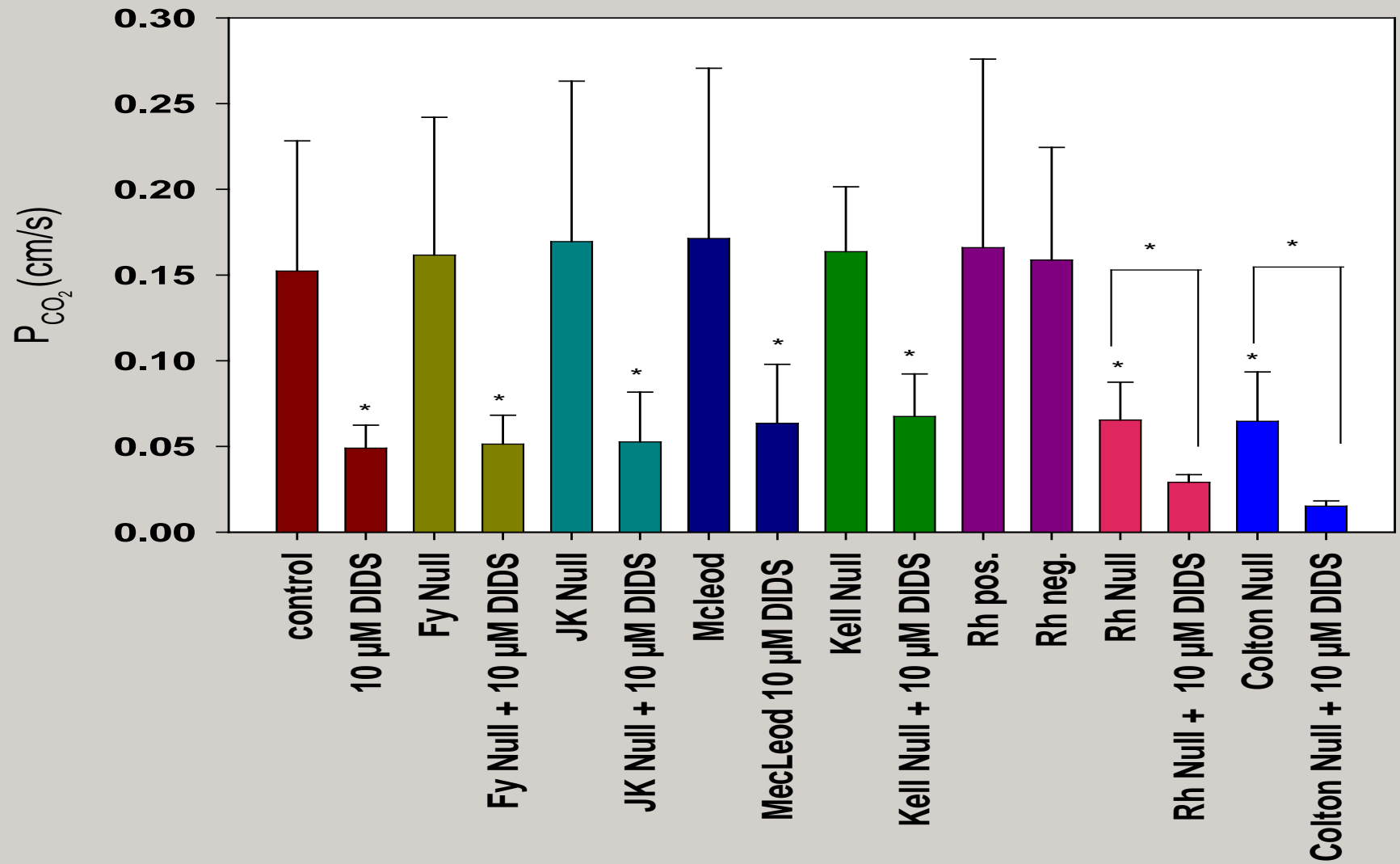
The method is applicable to suspensions of isolated cells or vesicles and to intact epithelia

Intrinsic CO₂ permeability of cell membranes and role of CO₂ channels

Volker Endeward, Fabian Itel, Samer Al-Samir,
Mohamed Chami, Fredrik Öberg, Kristina Hedfalk,
Gerolf Gros



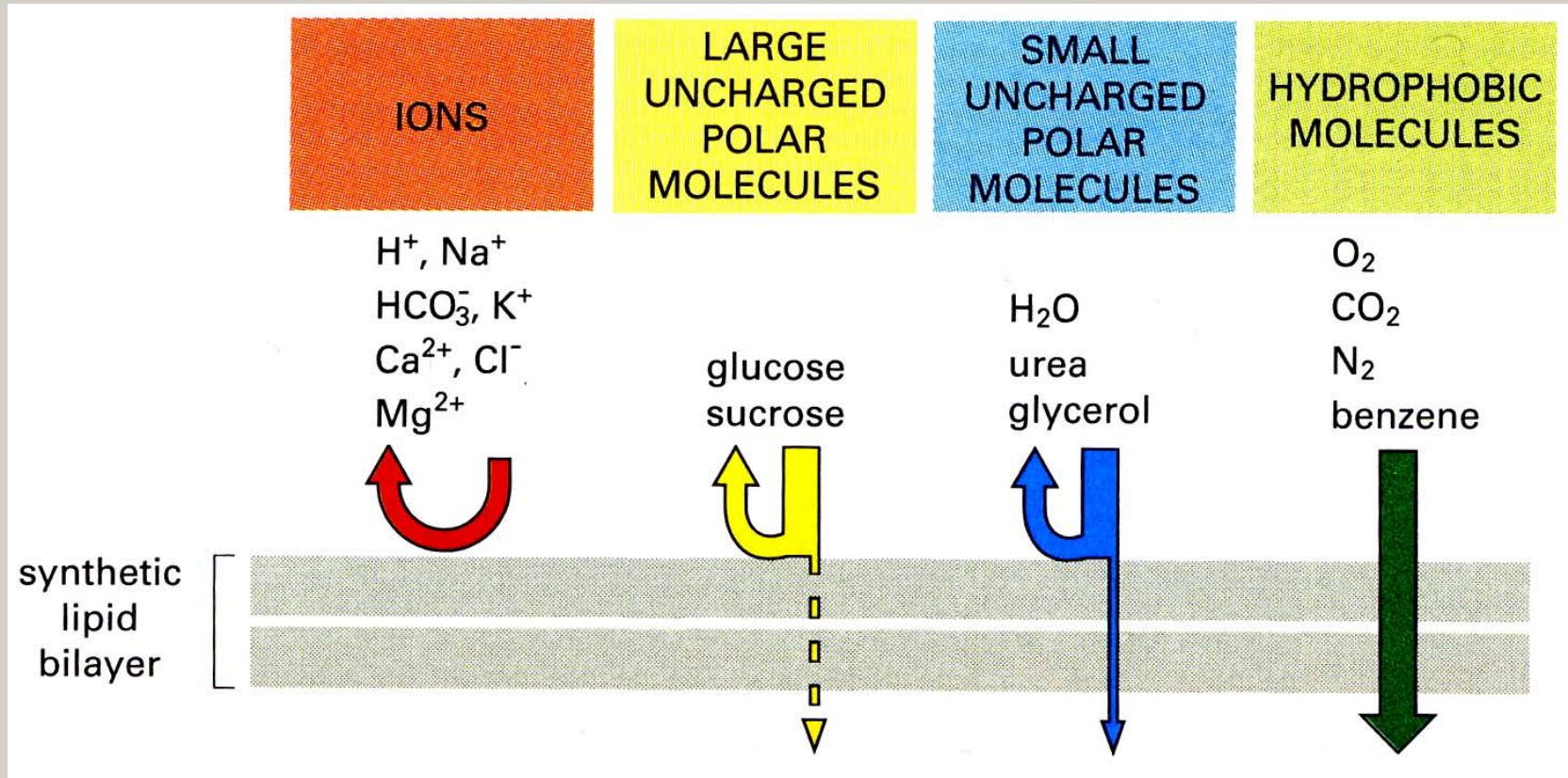
Medizinische Hochschule
Hannover



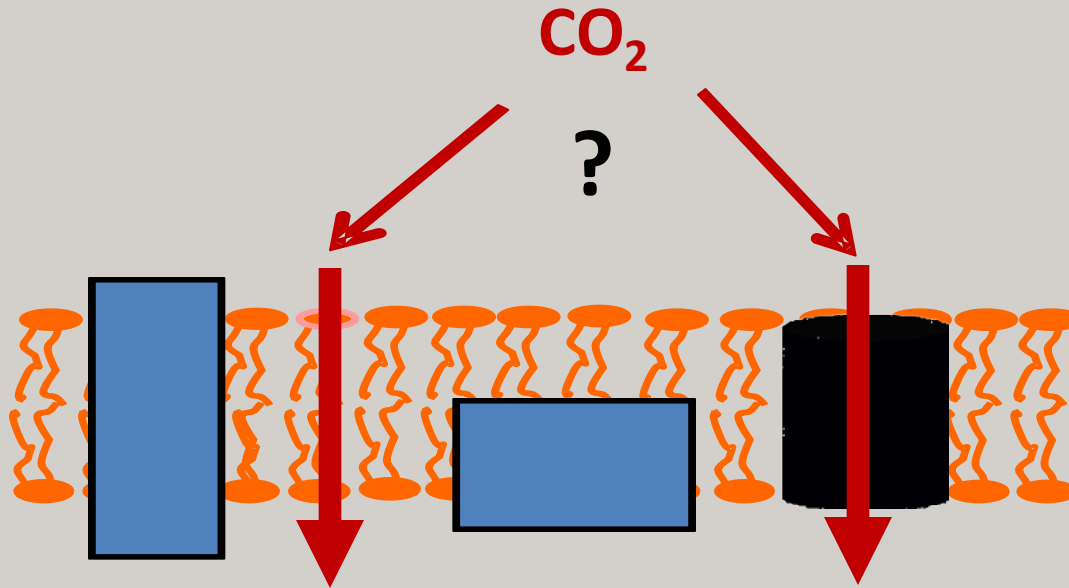
Intrinsic CO₂ permeability of a red cell membrane

	P_{CO2} (cm/s)
Red cell	0.15 ± 0.08
Red cell Ø AQP1, Ø functional Rh	0.015 ± 0.003

Gas permeability of synthetic phospholipid bilayers



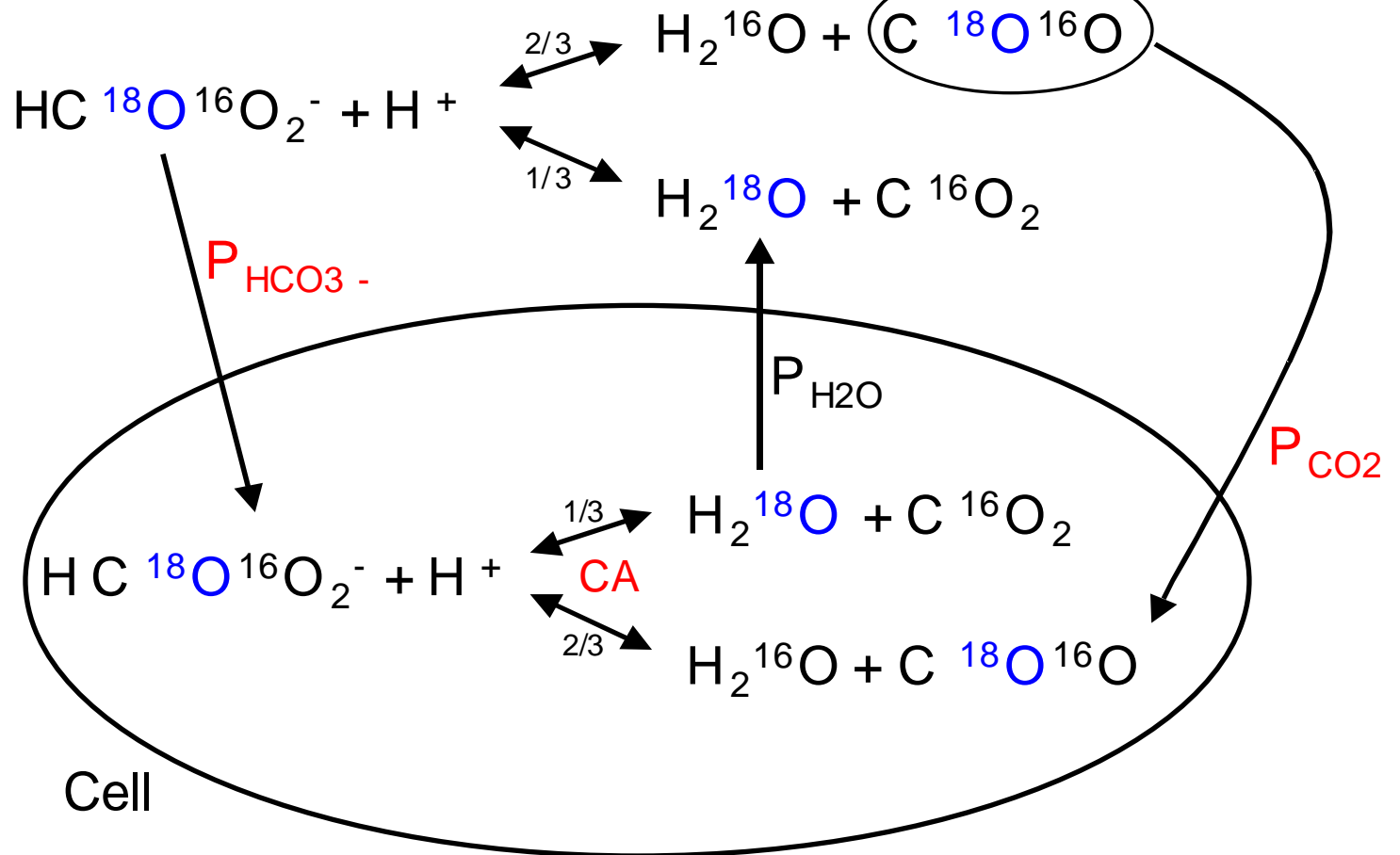
Alberts et al.
Molecular Biology Of The Cell, 4th Edition



1. What are the intrinsic CO_2 permeabilities of cell membranes?
2. Which mechanisms are responsible for the given intrinsic permeabilities of cell membranes?

1. What are the intrinsic CO₂ permeabilities of cell membranes?

Mass spectrometer



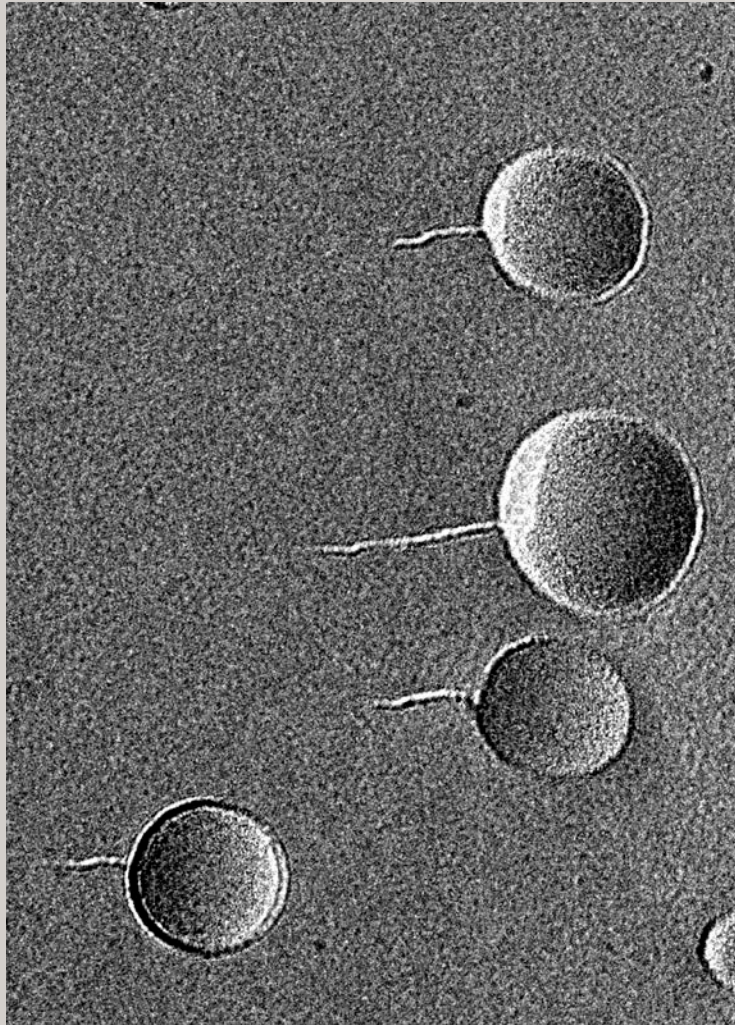
Cell membranes show CO₂ permeabilities lower than synthetic lipid bilayer

	P_{CO2} (cm/s) ± S.D.
Synthetic lipid bilayer	0.35 - 3.2
Red cell, Ø functional gas channel	0.015 ± 0.003
MDCK	0.017 ± 0.004
tsA201	0.007 ± 0.003
Basolateral membrane of proximal colon epithelium	~ 0.022
Apical membrane of proximal colon epithelium	0.0015 ± 0.0006

2. Which mechanisms are responsible for the given intrinsic permeabilities of cell membranes?

Parameter studied	Cholesterol fraction of total bilayer lipids (mol %)	Ratio of parameter w over w/o cholesterol	
P_{NH_3}	30 %	0.31	Antonenko et al. 1997
P_{NH_3}	52 %	0.012	Hill & Zeidel 2000
$P_{\text{H}_2\text{O}} \text{ (f)}$	40 %	0.18	Lande et al. 1995
$P_{\text{H}_2\text{O}} \text{ (f)}$	52 %	0.026	Hill & Zeidel 2000
$P_{\text{H}_2\text{O}} \text{ (d)}$	66 % (L+Chol)	0.26	Finkelstein 1976
"	66 % (SM + Chol)	0.037	"

PC:PS:Chol – vesicles with different cholesterol content

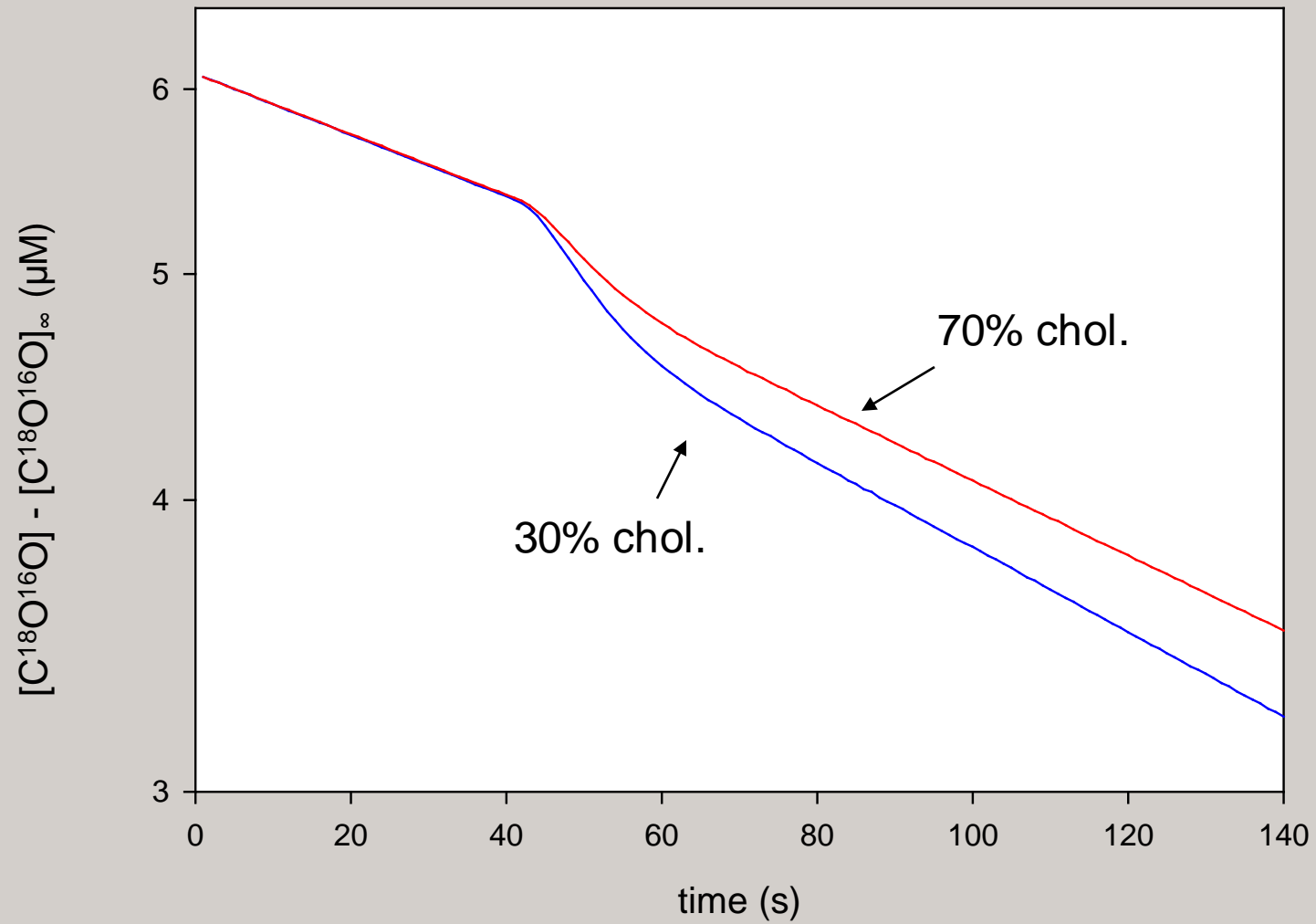


PC = Phosphatidylcholine

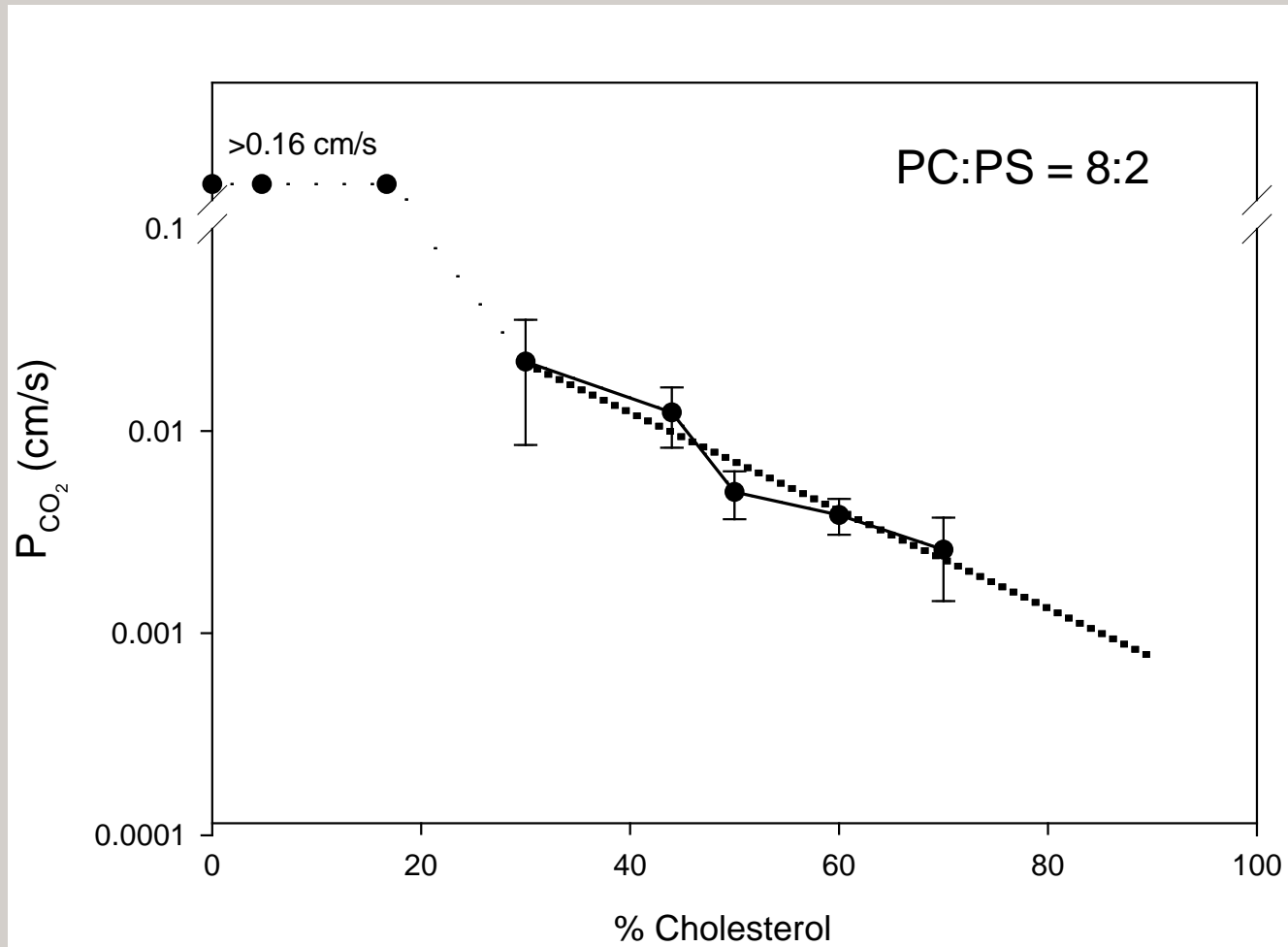
PS = Phosphatidylserine

Chol = Cholesterol (0 – 70%)

Ø = ~ 150 nm



Effect of cholesterol on lipid vesicle CO₂ permeability

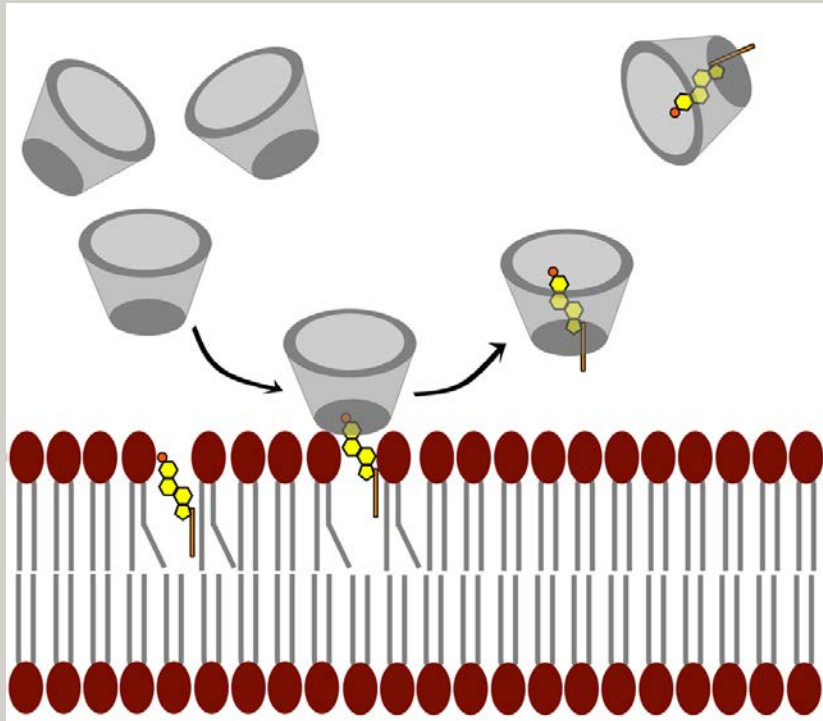


Comparison of cell membranes and cholesterol-containing vesicles

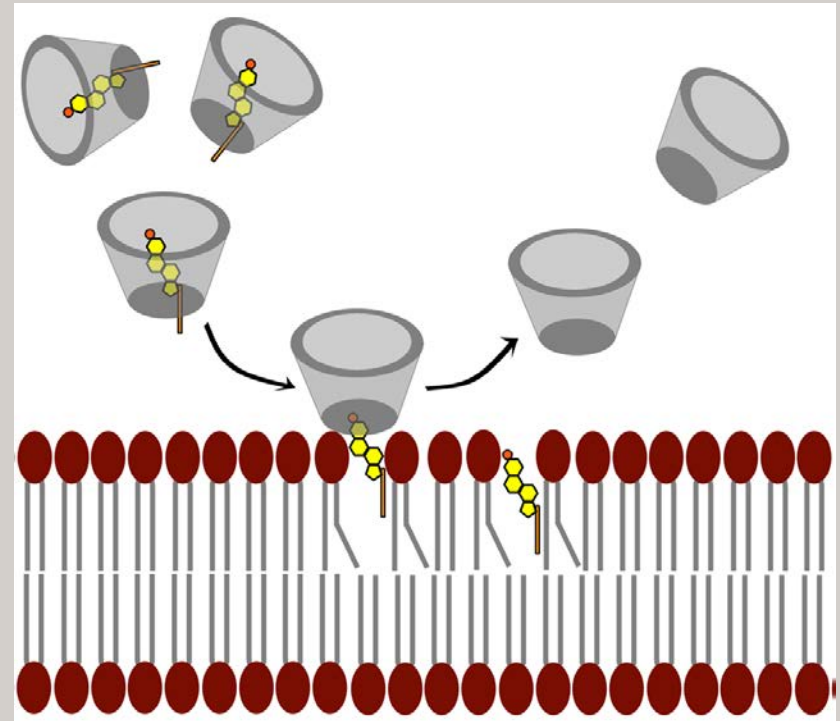
	$P_{\text{CO}_2} \text{ (cm/s)} \pm \text{S.D}$	Cholesterol content (Mol%)	P_{CO_2} predicted from cholesterol effect in vesicles (cm/s)
Lipid bilayer	0.35 / 3.2		-
Red cell: Ø AQP1, Ø functional Rh	0.015 \pm 0.003	45	0.010
MDCK	0.017 \pm 0.004	37	0.015
tsA201	0.007 \pm 0.003	-	-
Basolateral membrane prox colon epithelium	~ 0.022	42	0.011
Apical membrane of prox colon epithelium	0.0015 \pm 0.0006	77	0.0016

CO₂ permeabilities of cell membranes appear to be essentially determined by their cholesterol content

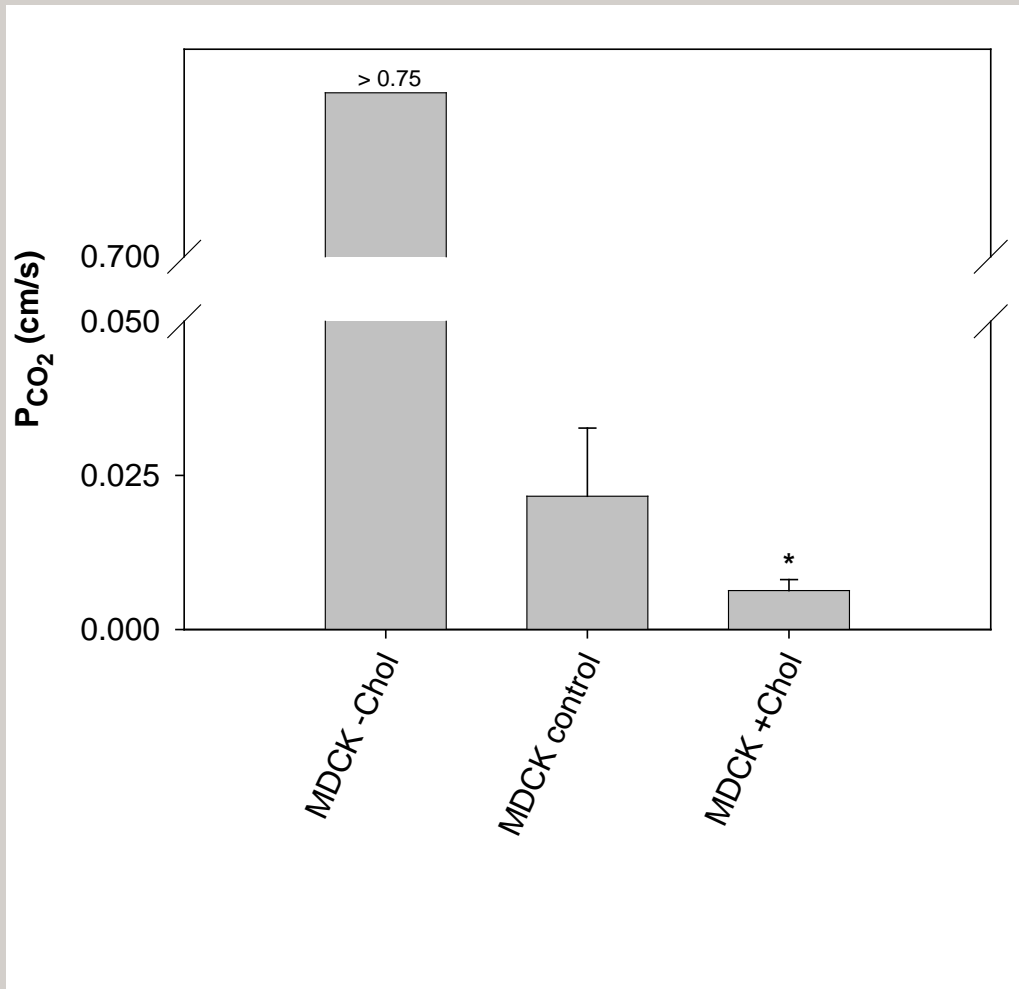
cholesterol depletion with β -cyclodextrin



cholesterol enrichment with β -cyclodextrin



Is cholesterol the cause of the low CO_2 permeability of MDCK cells?



Reduction of cholesterol with cyclodextrin raises P_{CO_2} .

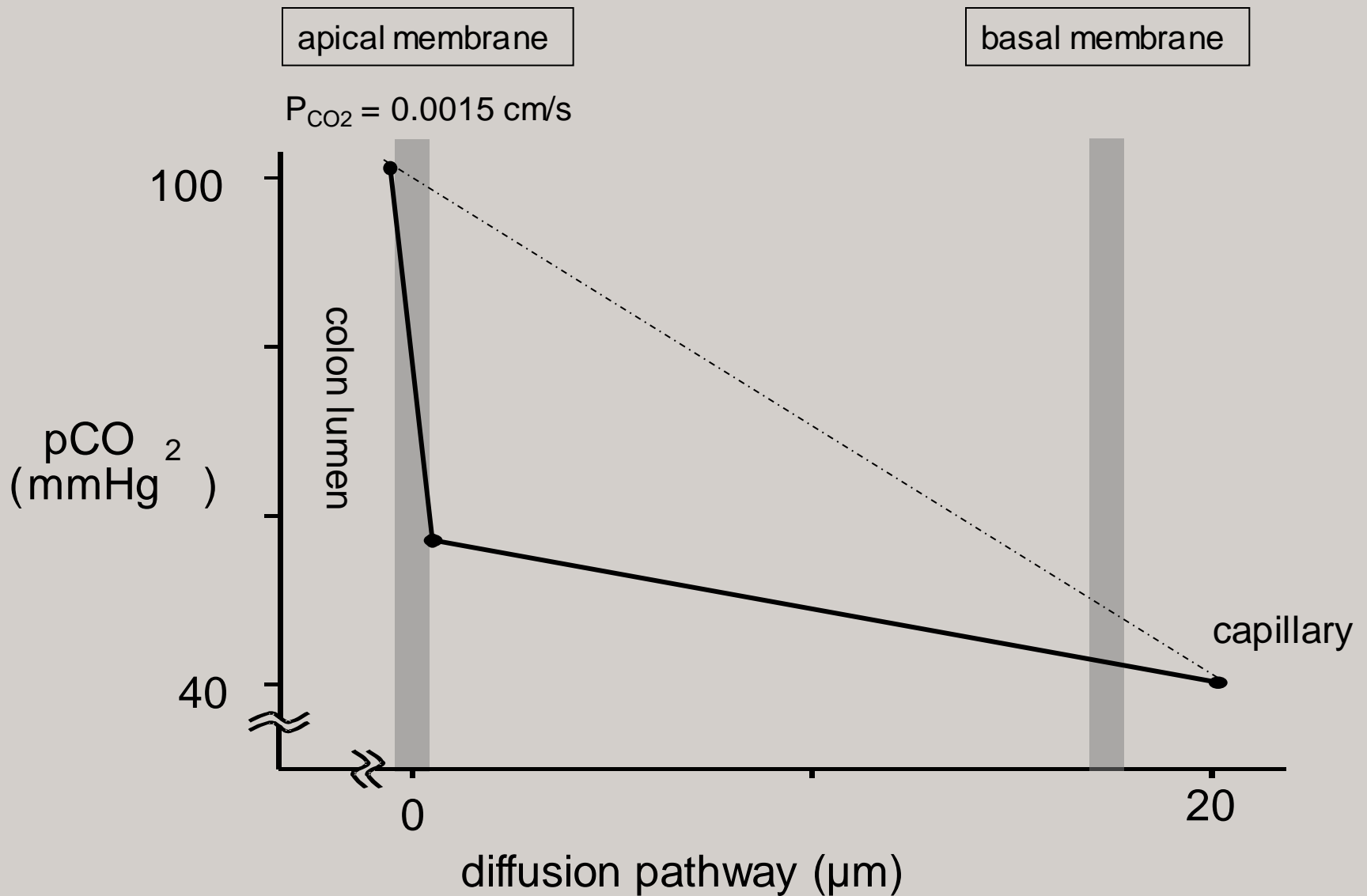
Enrichment with cholesterol lowers P_{CO_2} compared to normal cells.

- We show that cell membranes possess a low intrinsic CO₂ permeability, often in the range of 0.01 cm/s.
- This permeability is 2, and in one case 3, orders of magnitude lower than the CO₂ permeability of pure artificial phospholipid bilayers.
- The main cause of this low CO₂ permeability is the cholesterol content of the cell membrane. With increasing cholesterol content P_{CO_2} decreases in artificial vesicles as well as in intact cells.

Physiological consequences of low CO₂ membrane permeabilities

1. Consequences of the extremely low CO₂ permeability of the apical membrane of colon epithelium
2. Effect of low CO₂ membrane permeability on red blood cell gas exchange

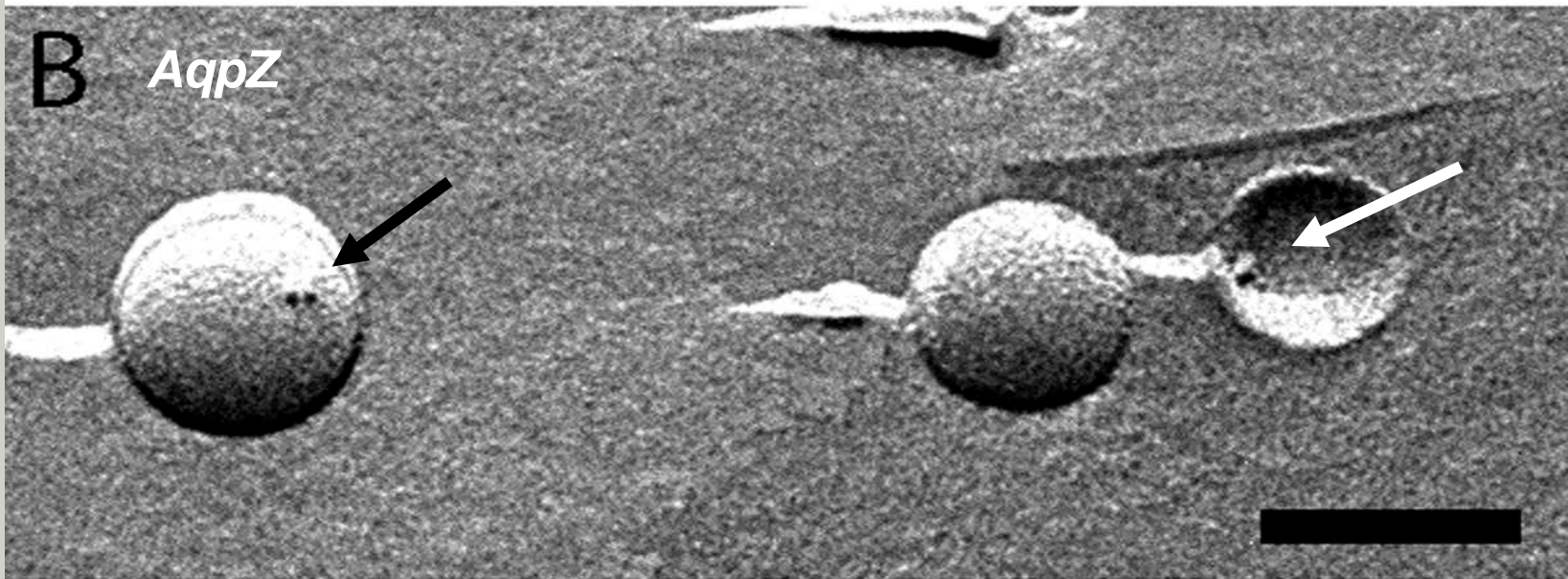
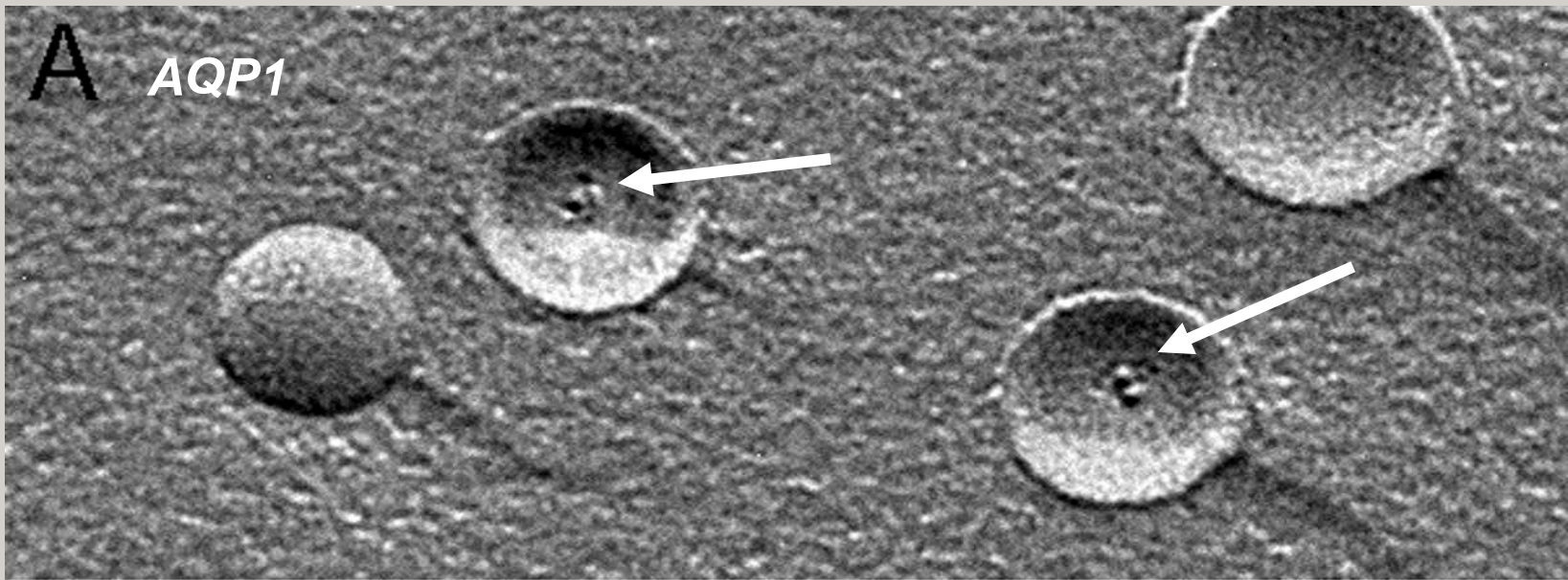
Consequences of low apical CO₂ permeability in colonocytes



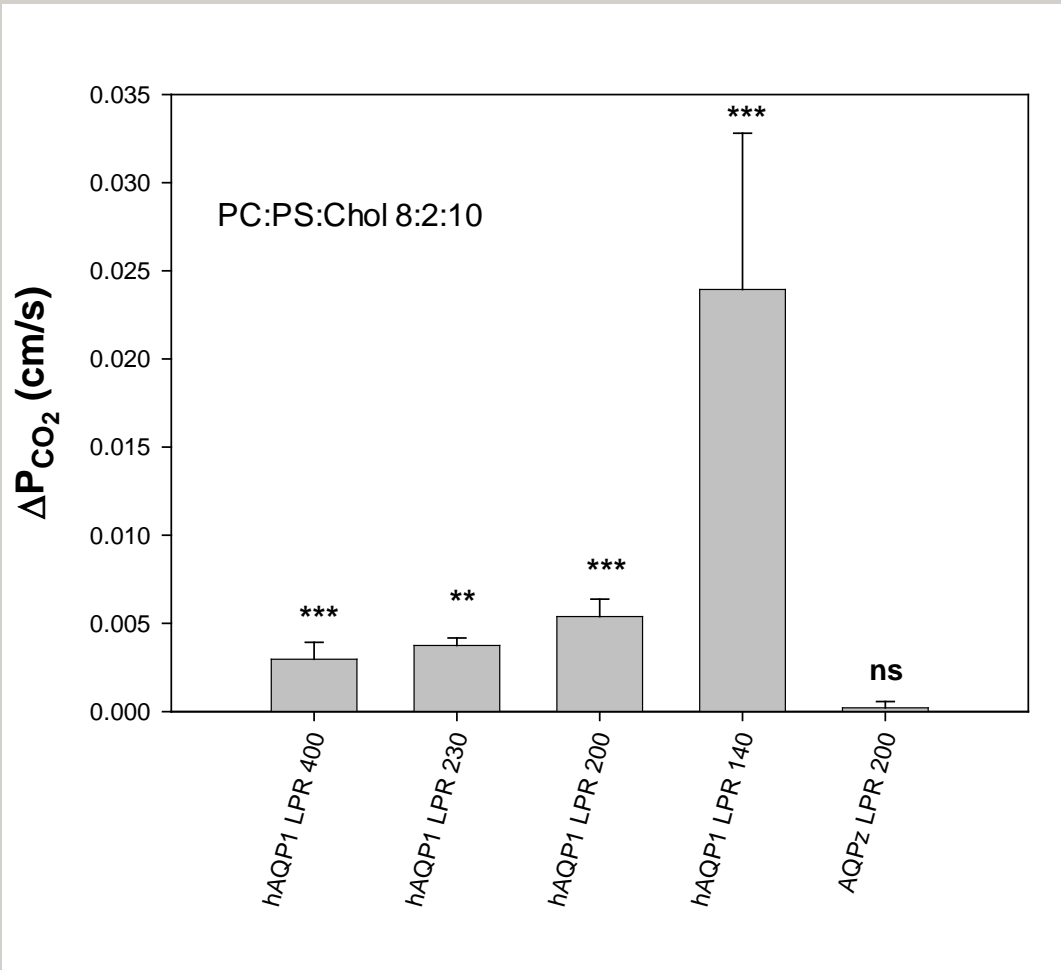
Example of a cell with a high gas exchange: red blood cell

	P_{CO2} (cm/s)	t₉₅ (ms)	transit time lung capillary (ms)	transit time heavy exercise (ms)
normal membrane resistance	0.15	110	700	350
permeability Ø functional gas channel	0.01	1000	700	350

- From these considerations we can see that gas exchange of cells with a low CO_2 permeability is limited.
- Hypothesis: cell membranes with normal cholesterol and low intrinsic P_{CO_2} adapt their CO_2 permeabilities to their needs by incorporating gas channels in the membrane.



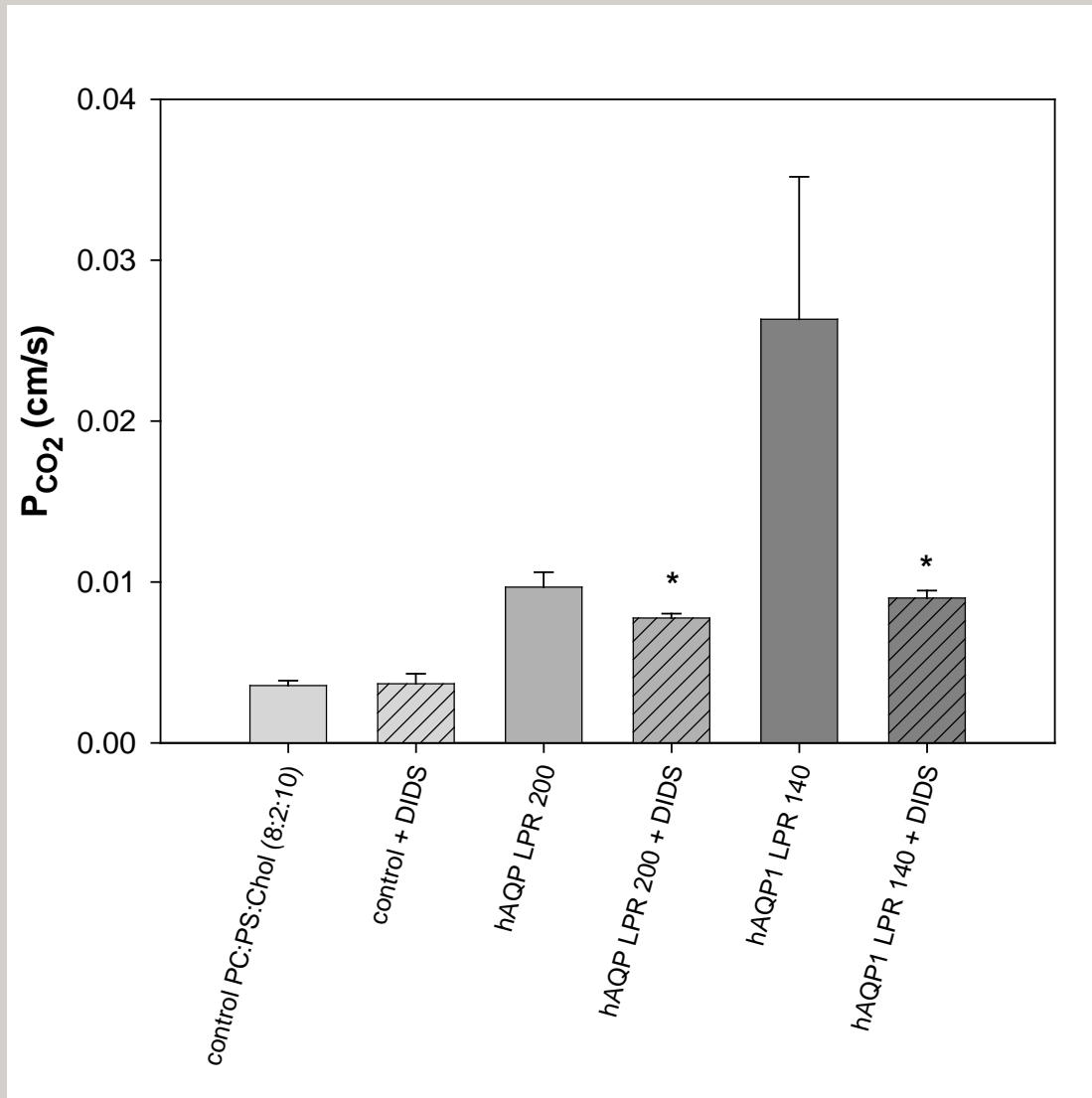
Aquaporin 1 as a CO₂ channel in cholesterol-containing lipid vesicles



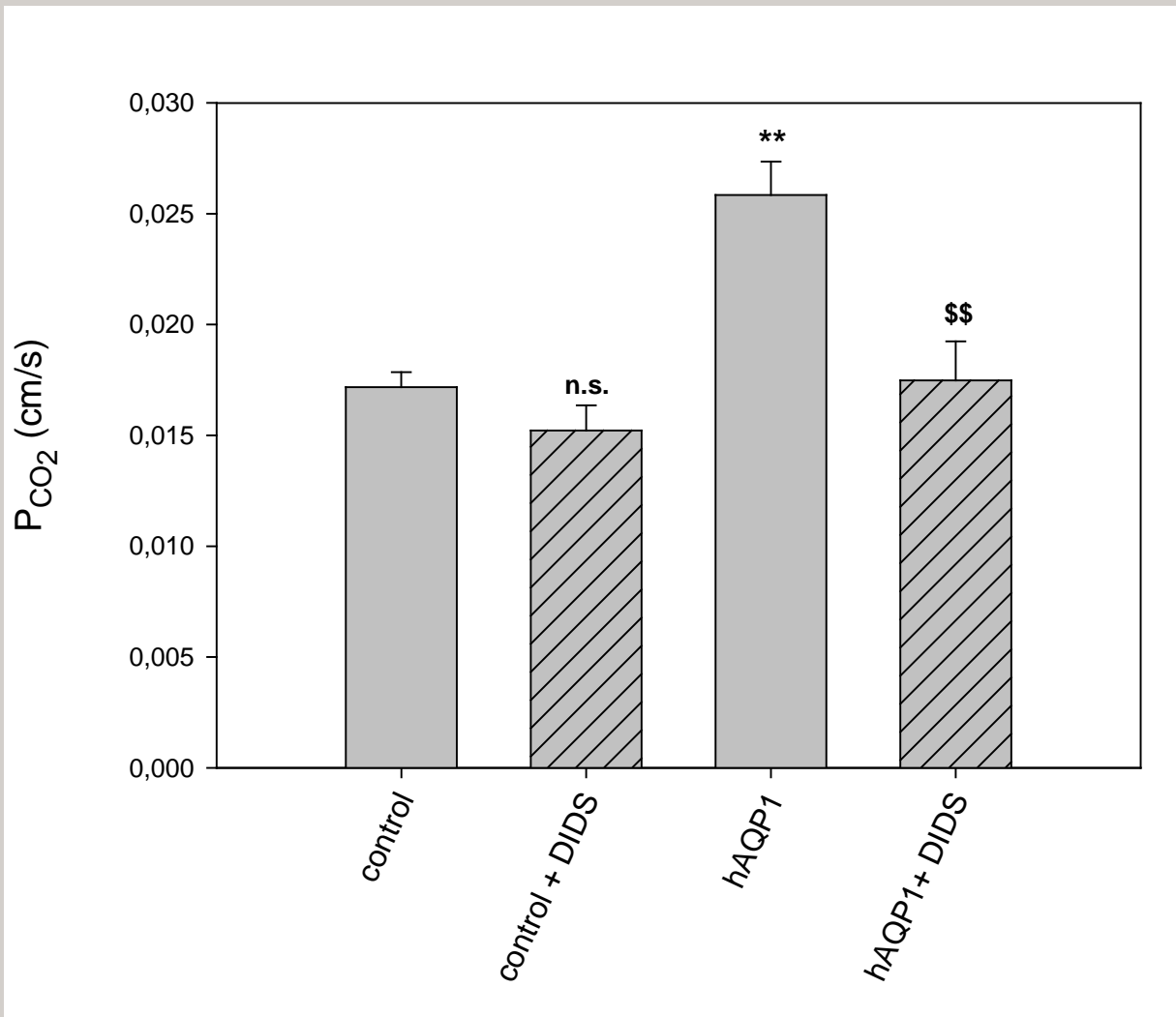
Incorporation of AQP1 into vesicles causes a rise in P_{CO₂}

Change of P_{CO₂} in vesicles with decreasing Lipid-Protein-Ratios (LPR)

DIDS reduces the CO₂ permeability of AQP1 containing vesicles



Aquaporin 1 as a CO₂ channel in MDCK cells



Expression of AQP1 in MDCK cells raises P_{CO_2}

- We conclude that in a membrane of normal cholesterol content and low CO₂ permeability, incorporation of AQP1 into the membrane significantly increases the CO₂ permeability in a concentration dependent manner.
- AQP1 acts as a DIDS-sensitive CO₂ channel.

Gas	CO ₂	O ₂	NO	N ₂
Lipid-water partition coefficient	0.95	2.9	3.8	4.1

	CO₂	O₂
Lipid-water partition coefficient	0.95	2.9
Reduction of membrane permeability by cholesterol	1/100	(1/100) ?
Membrane permeability	0.01 cm/s	(0.03 cm/s) ?
Heart muscle under heavy exercise: partial pressure difference across the membrane ΔP	5 mmHg	(40 mmHg) ?

Summary

With rising cholesterol content the CO_2 permeability (P_{CO_2}) of lipid vesicles decreases drastically.

The intrinsic P_{CO_2} of cell membranes is low due to their cholesterol content:

- 1) cell membranes and lipid vesicles with identical cholesterol content exhibit identical CO_2 permeability
- 2) cholesterol-depleted cell membranes have an increased CO_2 permeability, cholesterol-enriched cell membranes a reduced permeability

Cell membranes with normal cholesterol raise their CO_2 permeability, when functionally required, by incorporation of CO_2 channels:

- 1) AQP1 incorporated in lipid vesicles raises CO_2 permeability in a concentration-dependent manner
- 2) AQP1 expression in MDCK cells increases membrane P_{CO_2} .

Medizinische Hochschule Hannover
Vegetative Physiologie

Samer Al-Samir

Timo Meine

Werner Zingel

Gerolf Gros

Universität Basel
Dept. Chemie / Biozentrum

Fabian Itel

Mohamed Chami

University of Gothenburg
Dept. Chemistry/Biochemistry

Frederic Öberg

Kristina Hedfalk



Involvement of elevated membrane cholesterol on G- protein regulated water and gas transport in biological membranes

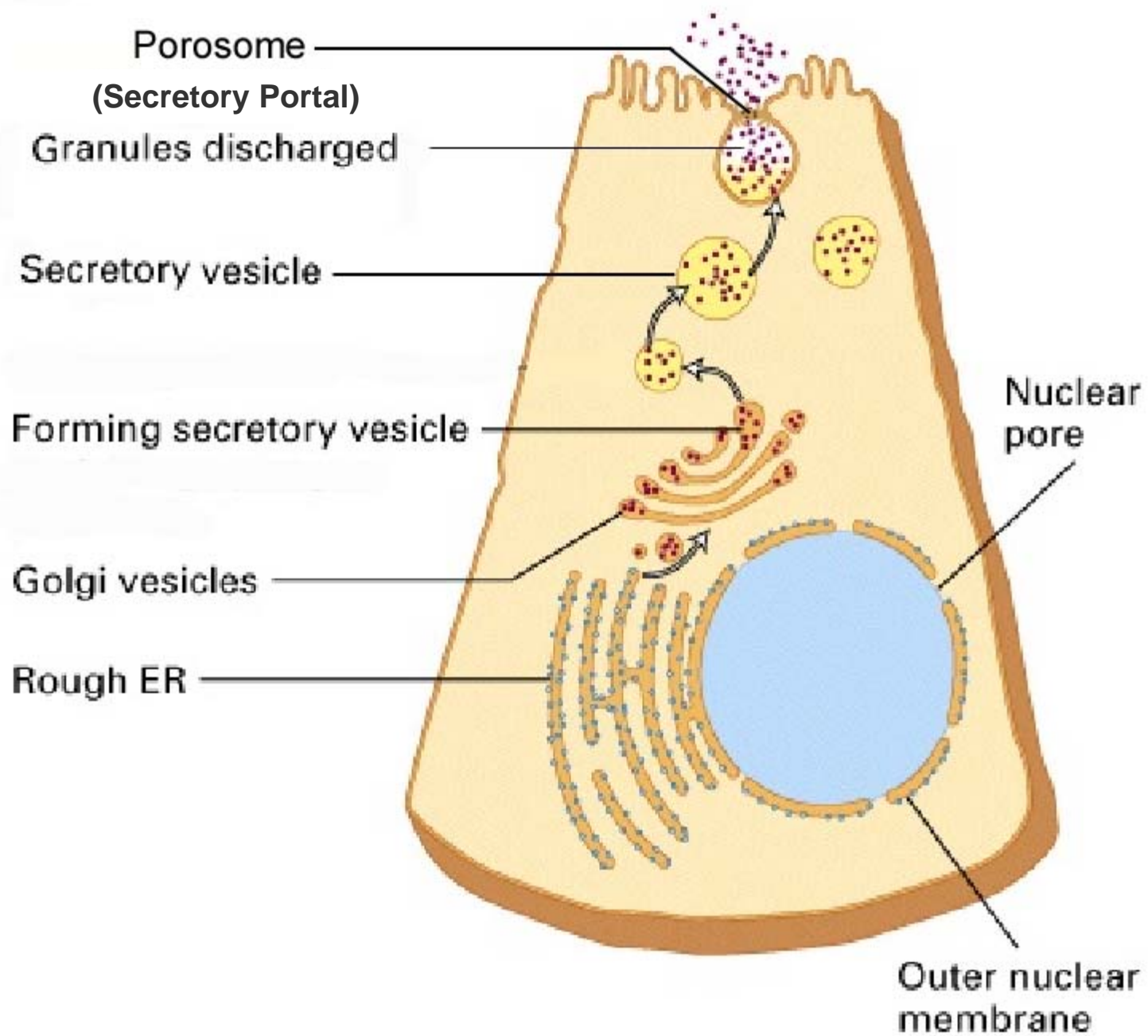
Gas Channels Workshop, Case Western Reserve University, OH; Sept. 5-7, 2012

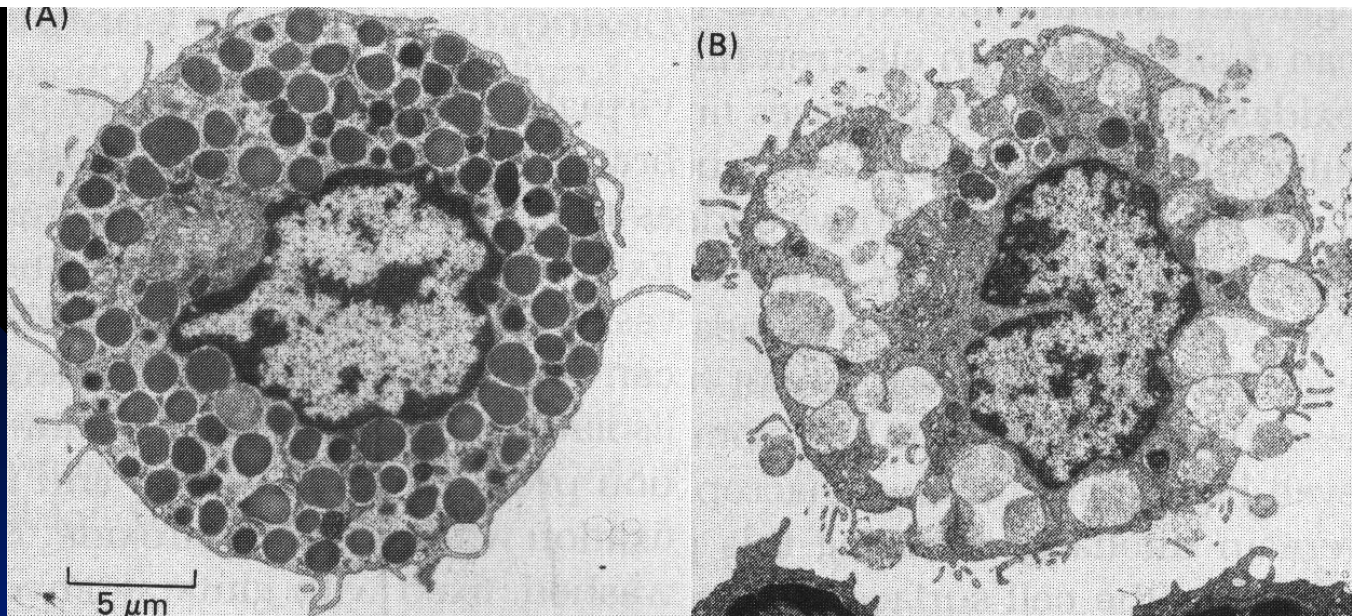
**Bhanu P. Jena
Dept. of Physiology
Wayne State University School of Medicine
Dept. of Chemical Engineering & Material Science
School of Engineering
Wayne State University
Detroit, MI 48201**



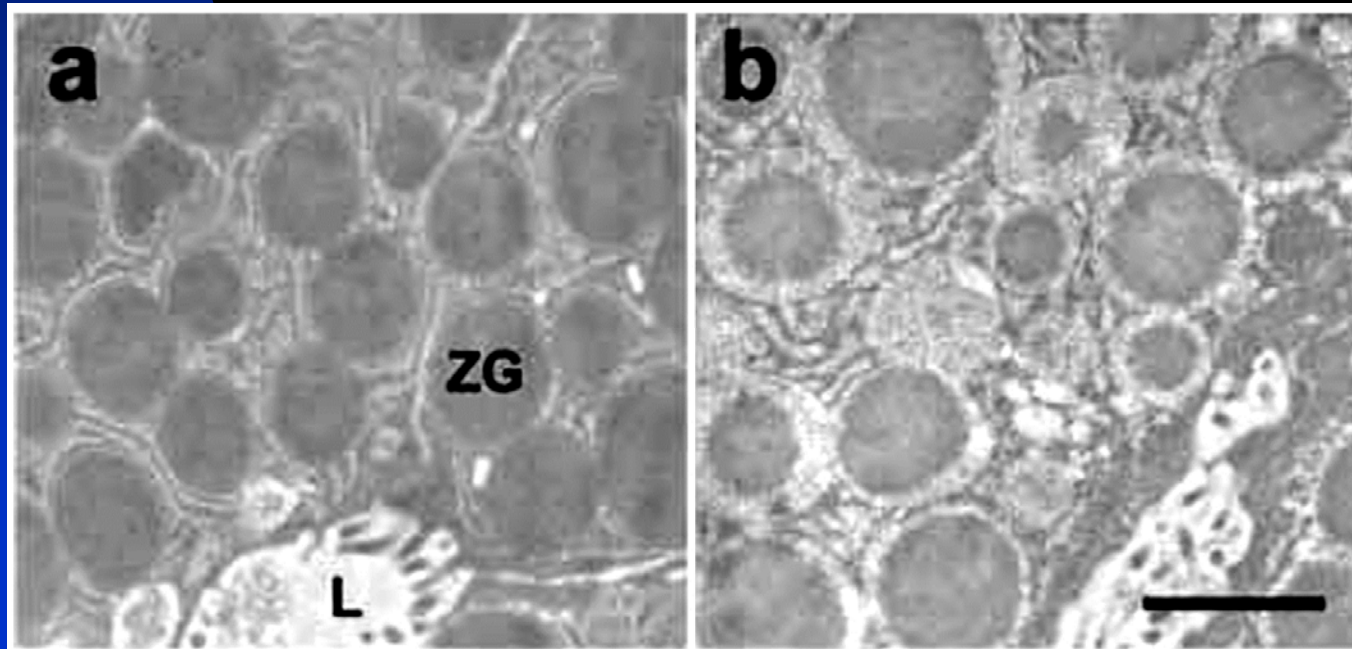
Jena Research Group





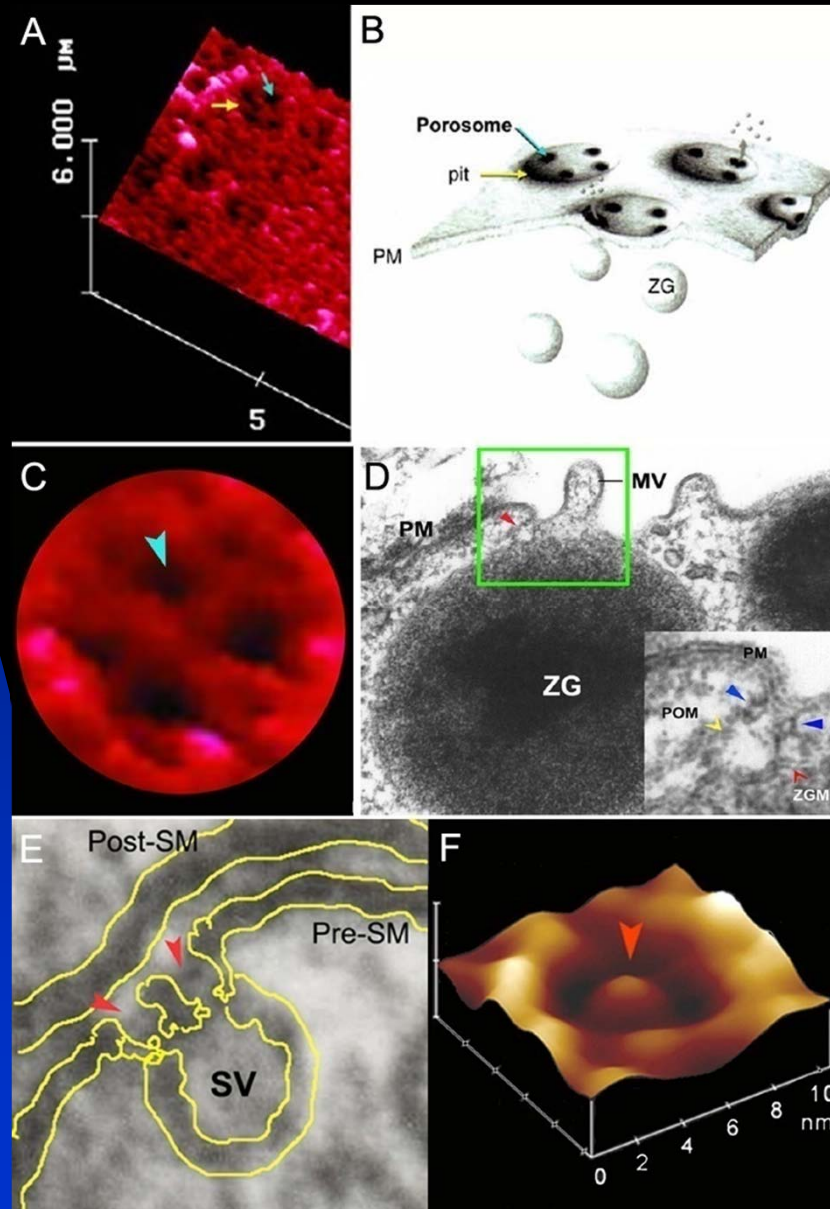


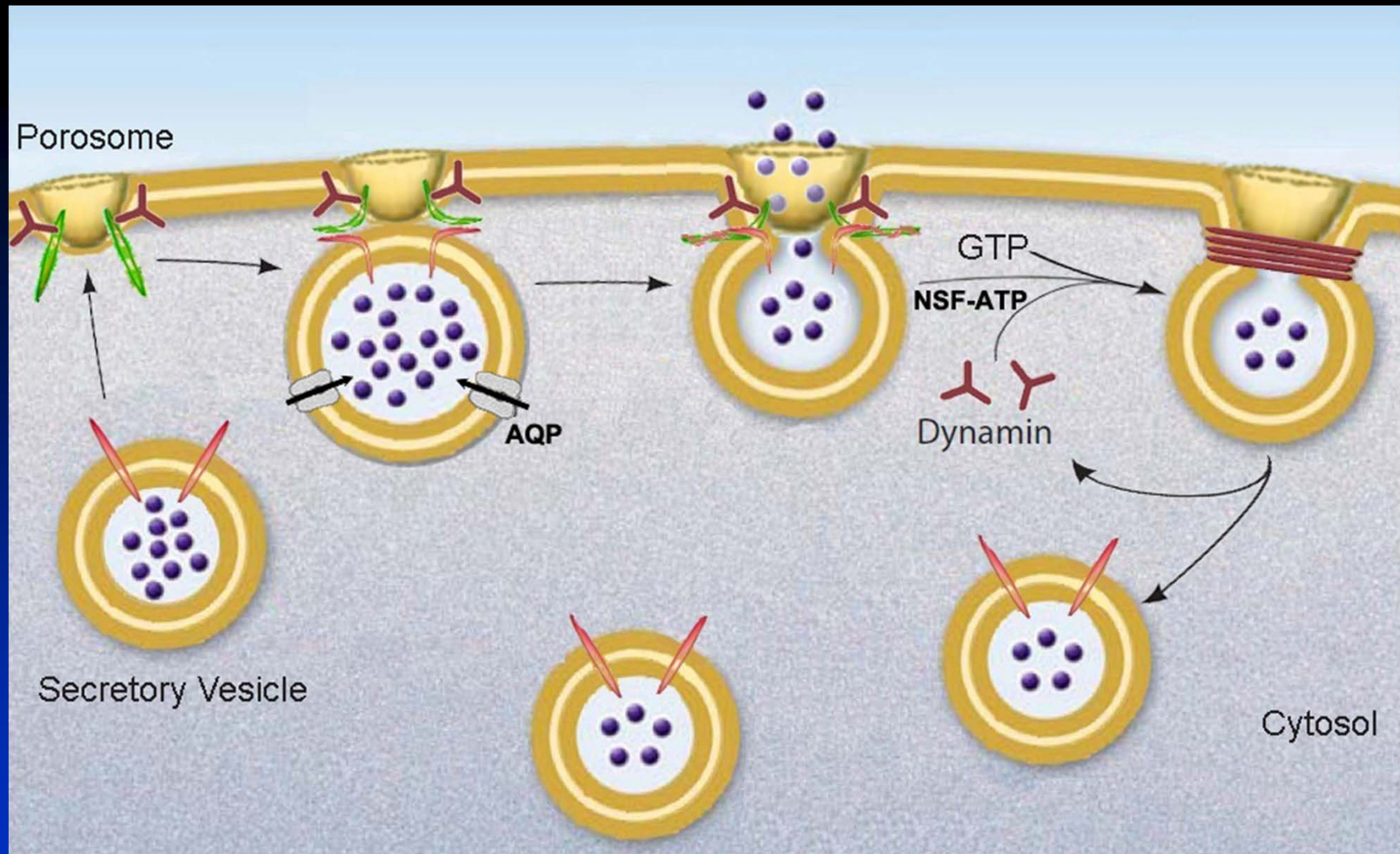
Rat Peritoneal Mast Cell- Lawson D. et al. 1975 *J. Exp. Med.* 142:391-402



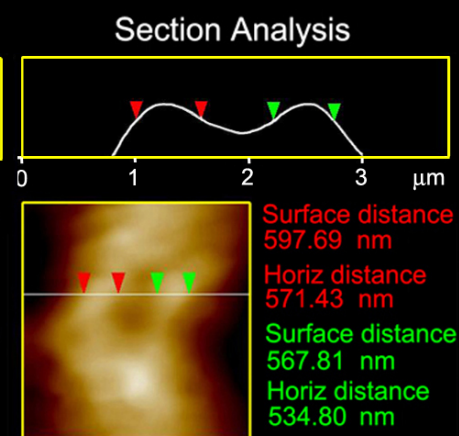
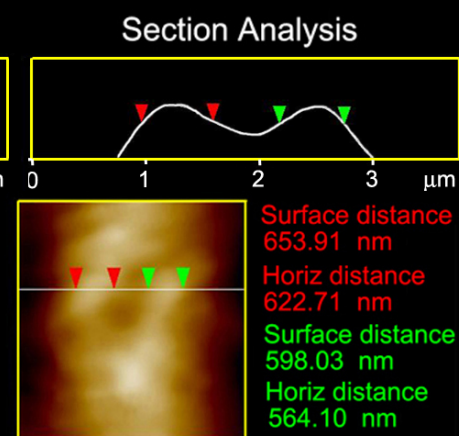
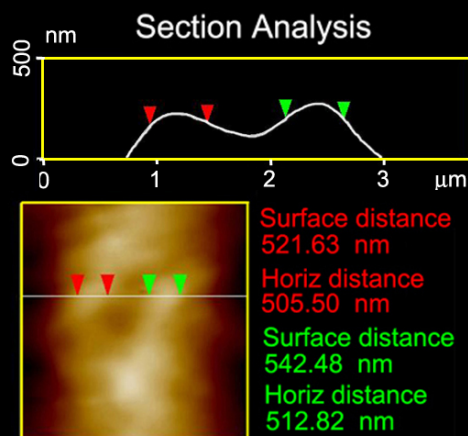
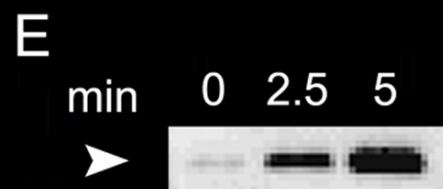
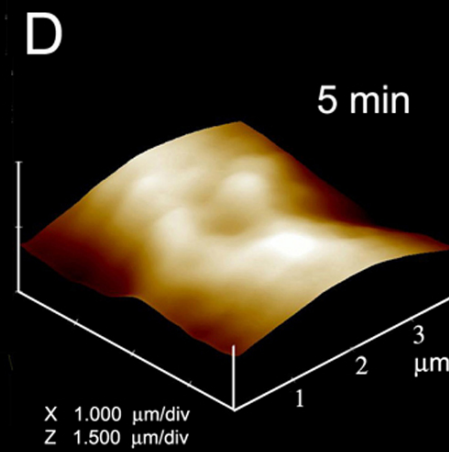
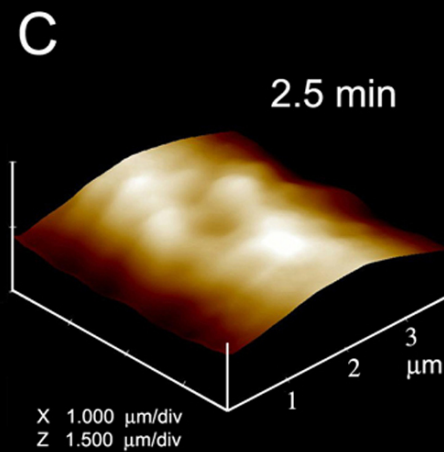
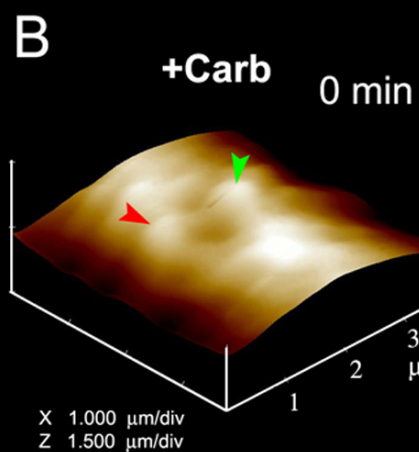
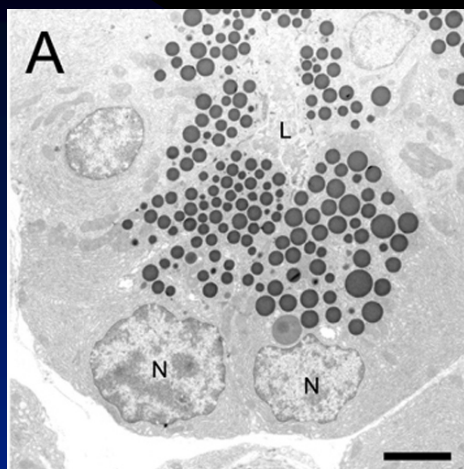
Rat Pancreatic Acinar Cells- Cho SJ et al. 2002 *Cell Biol. Int.* 26:29-33

Porosome: The Universal Secretory Portal in Cells

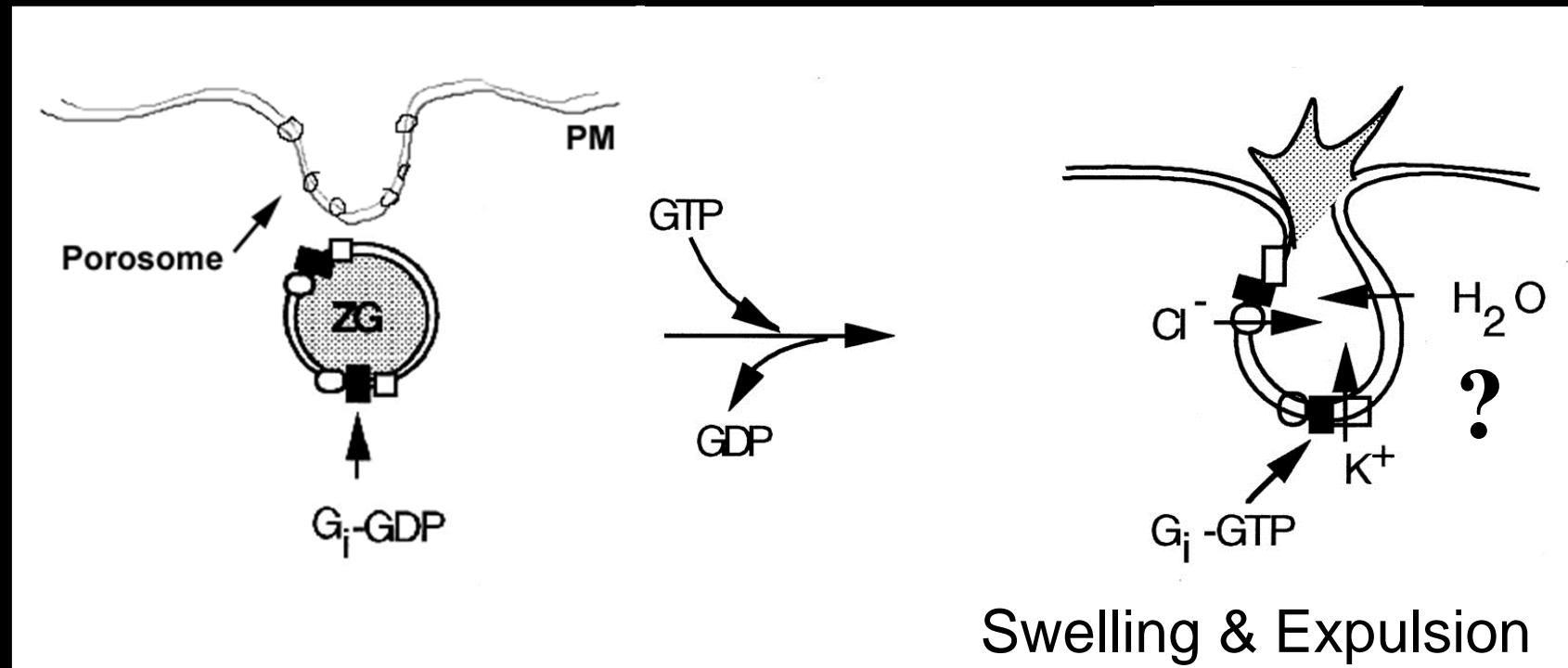




Lee et. al. 2012 J. Proteomics 75:3952-62

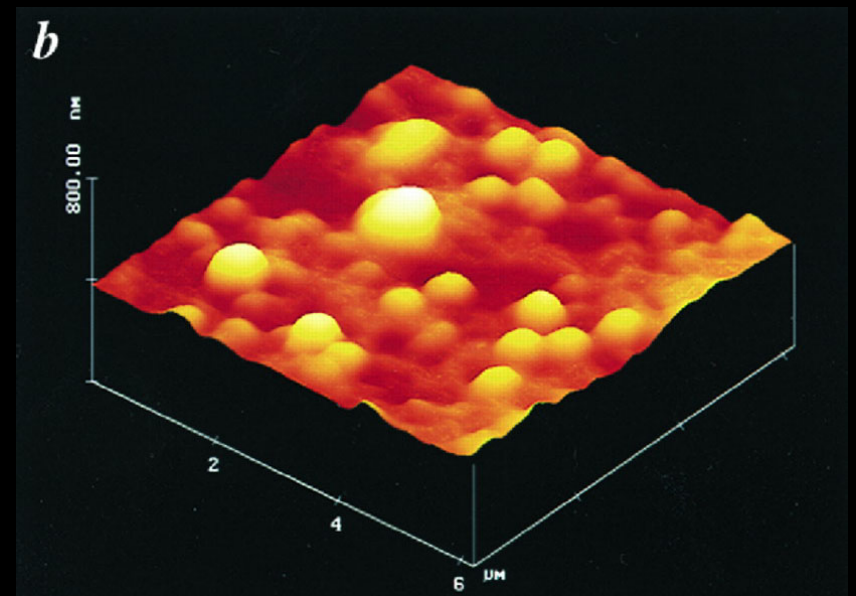
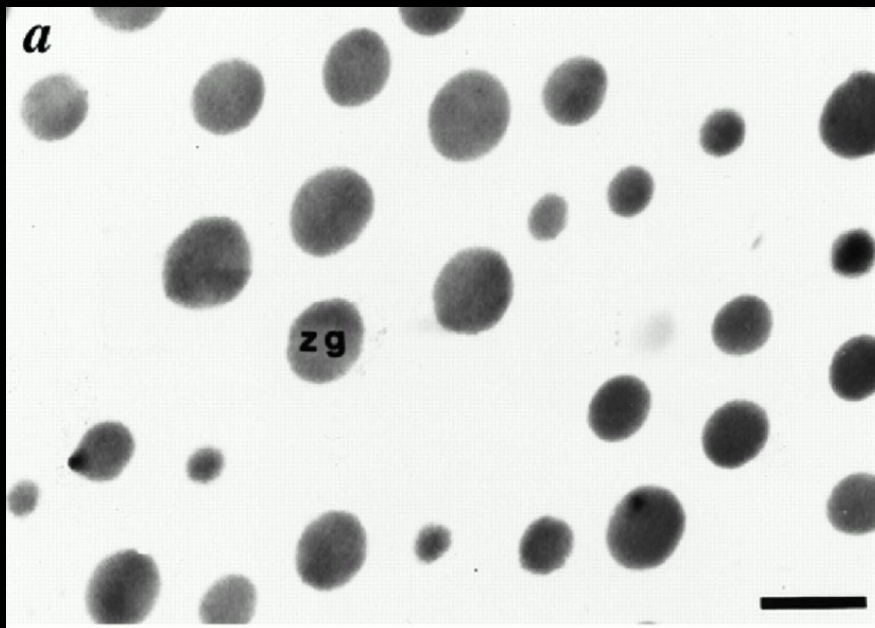


Hypothetical model



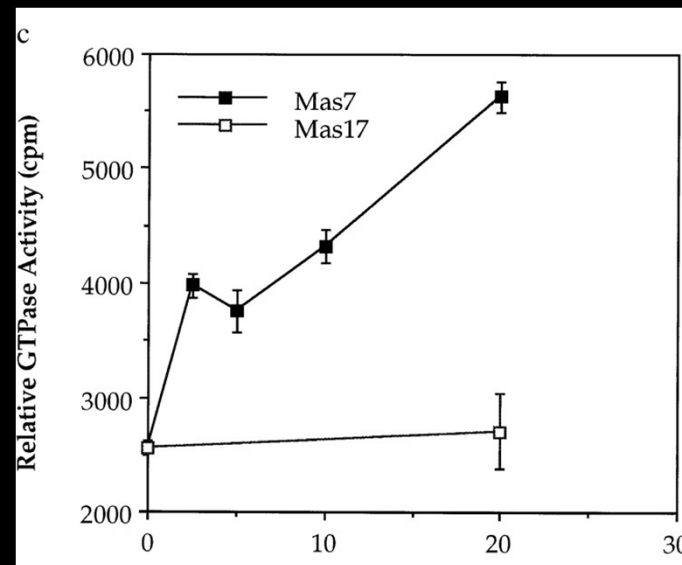
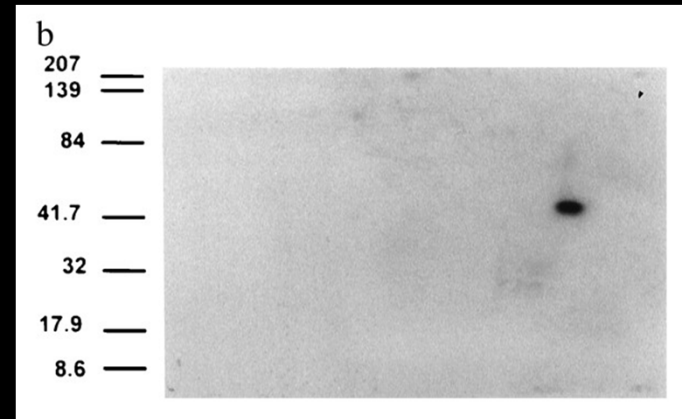
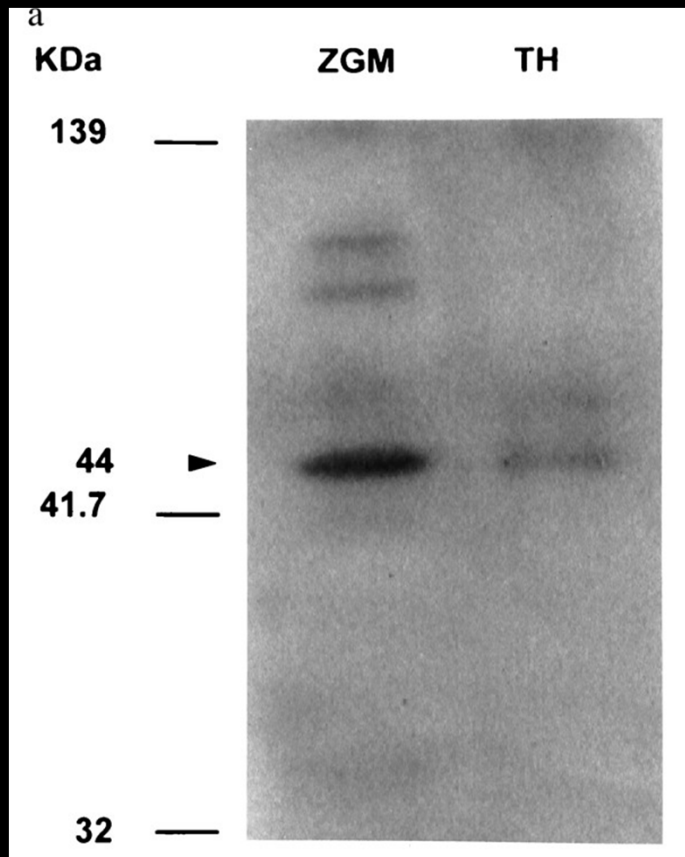
Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

EM & AFM micrographs of Zymogen Granules



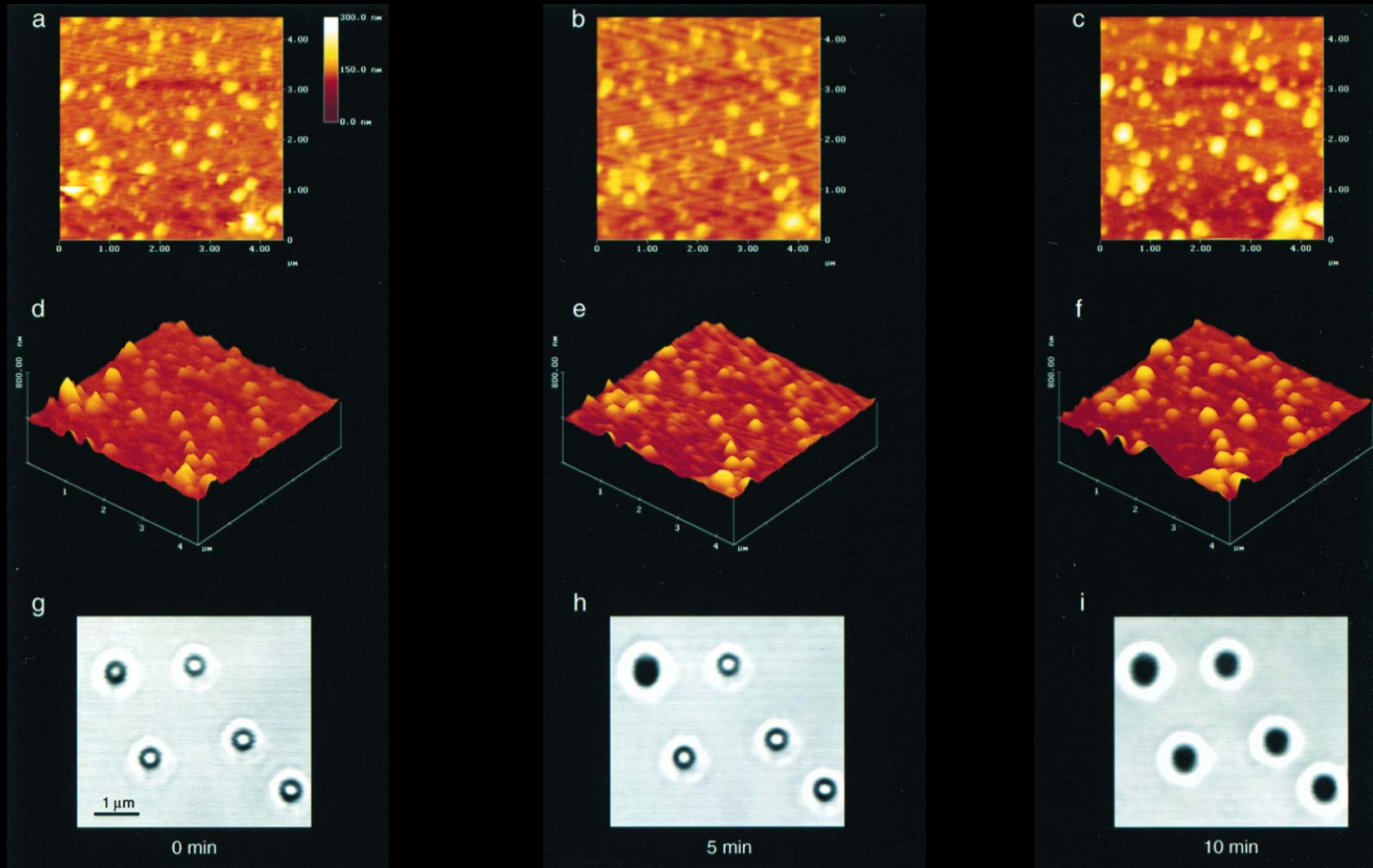
Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

Association of $G_{\alpha i3}$ with ZGM

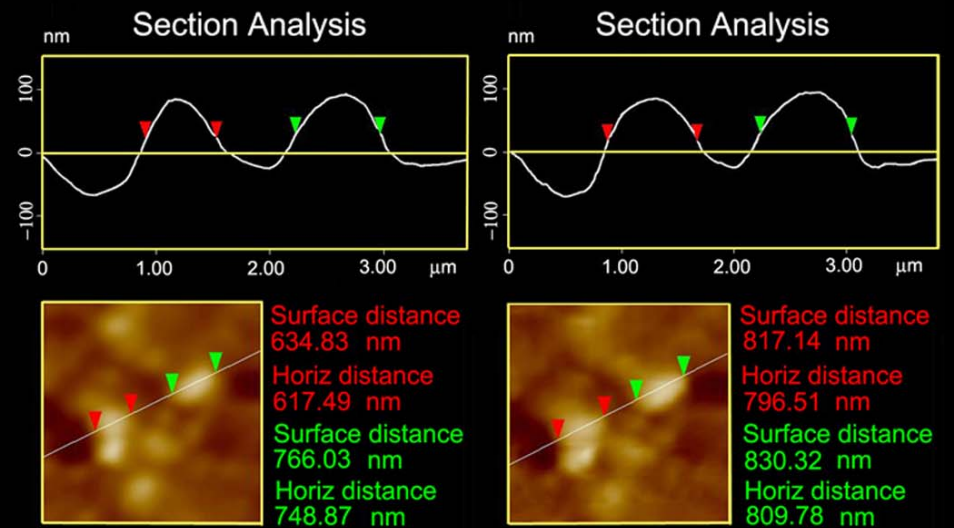
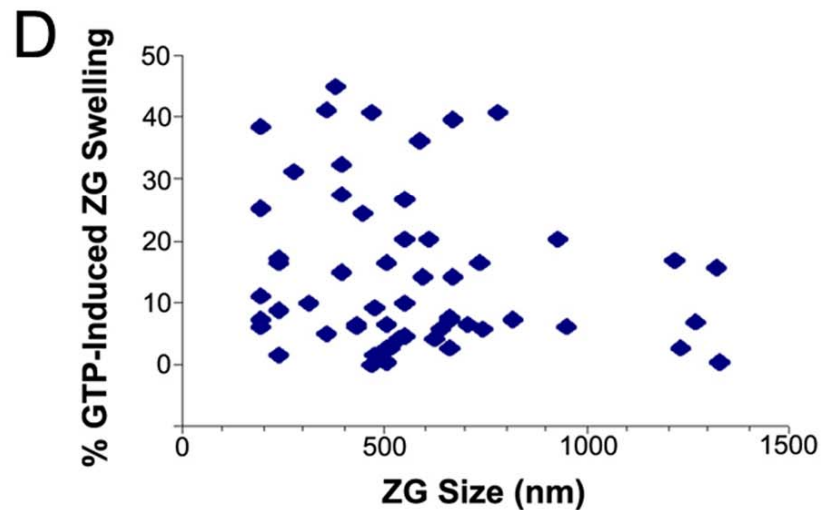
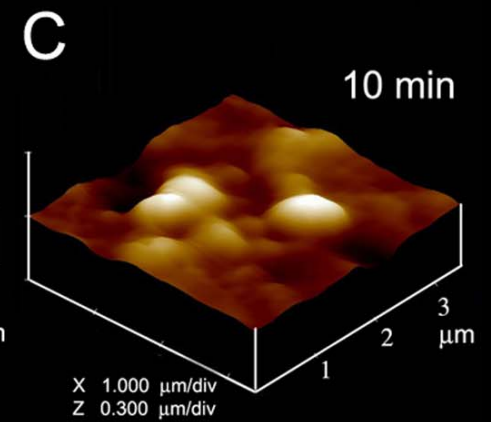
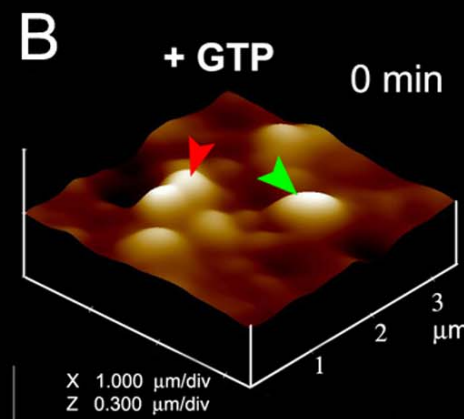
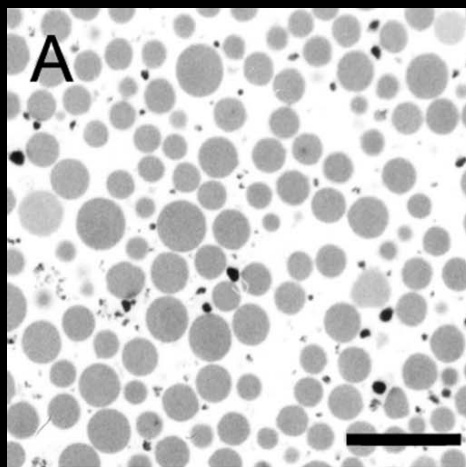


Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

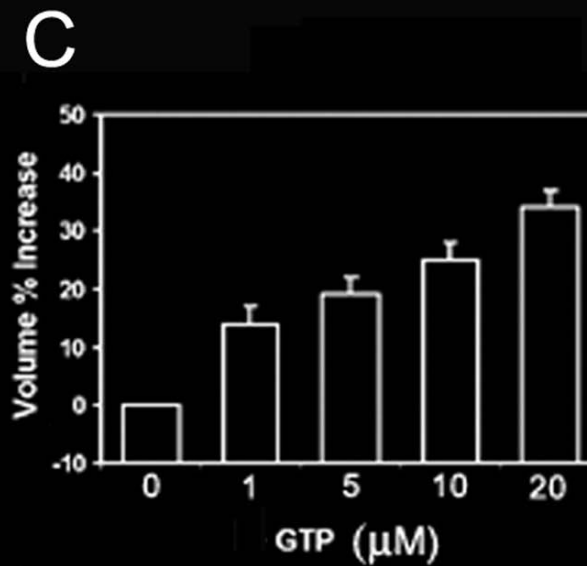
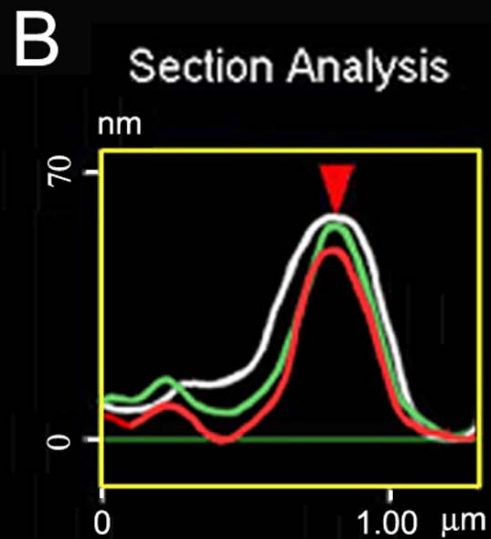
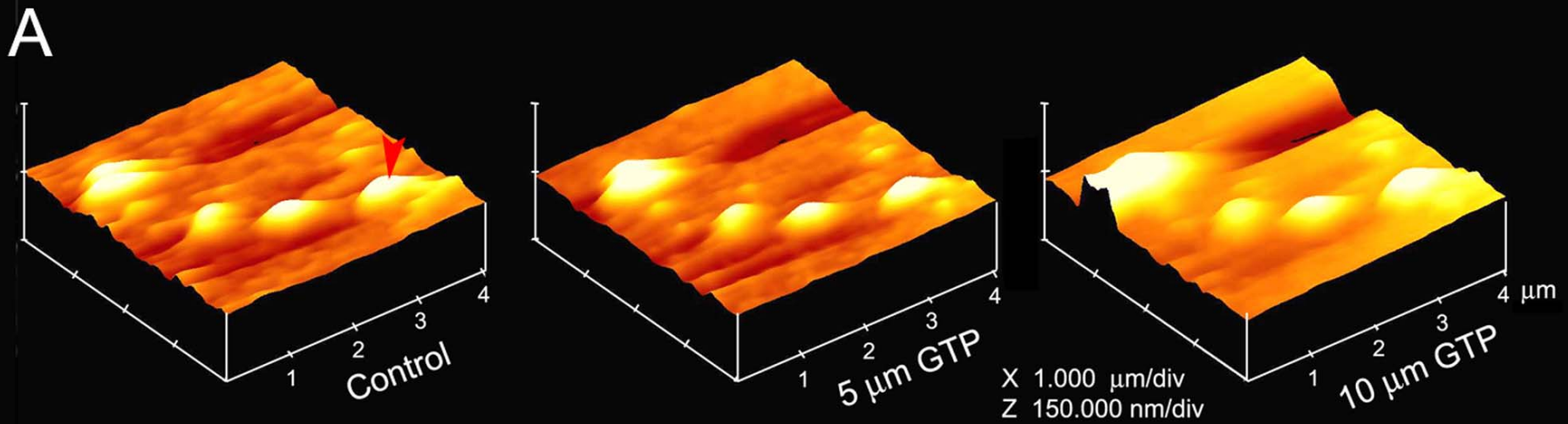
Vesicle Size Increase After Exposure to GTP



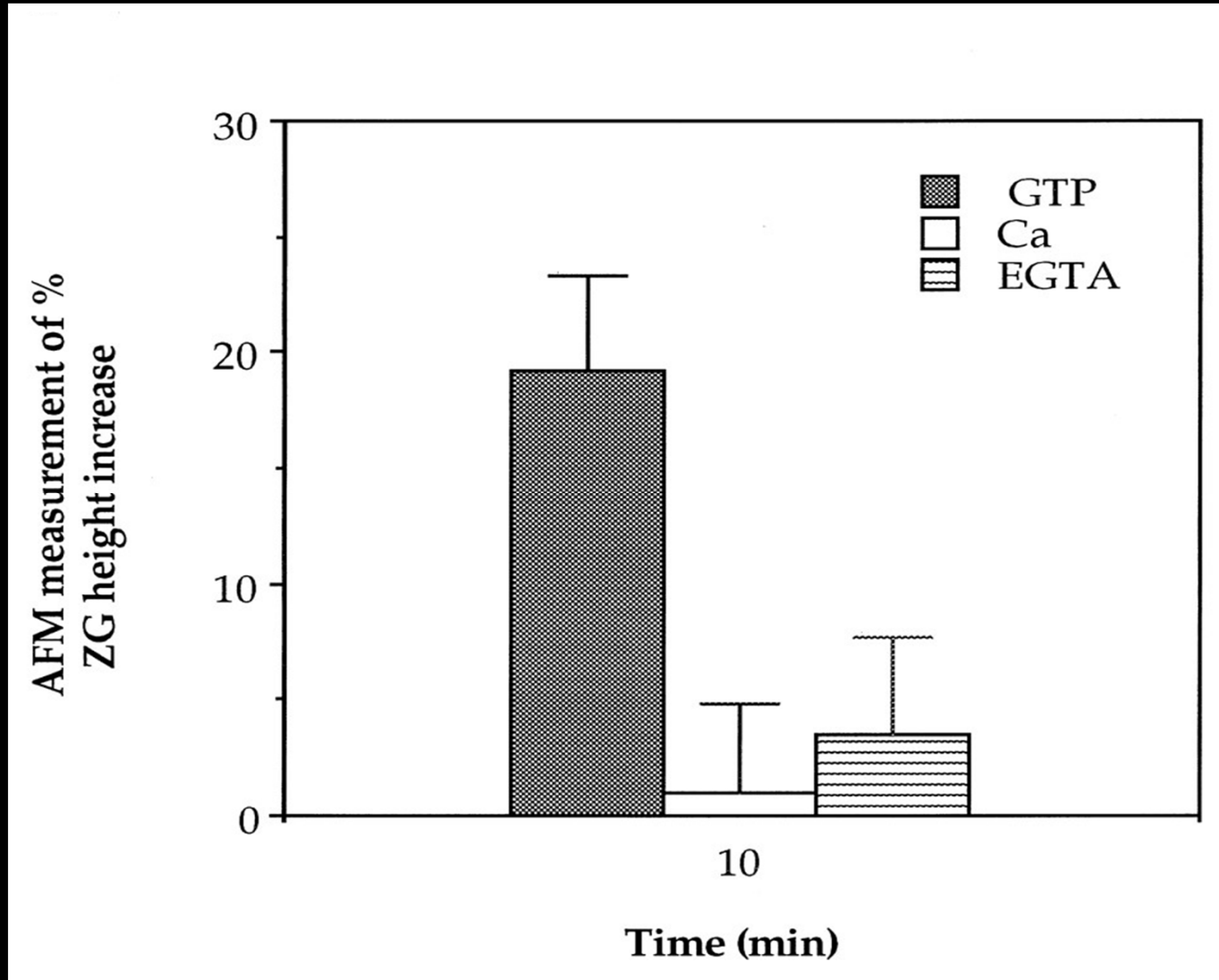
Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22



Kelly et. al. 2004 Cell Biol. Int. 28:709-16

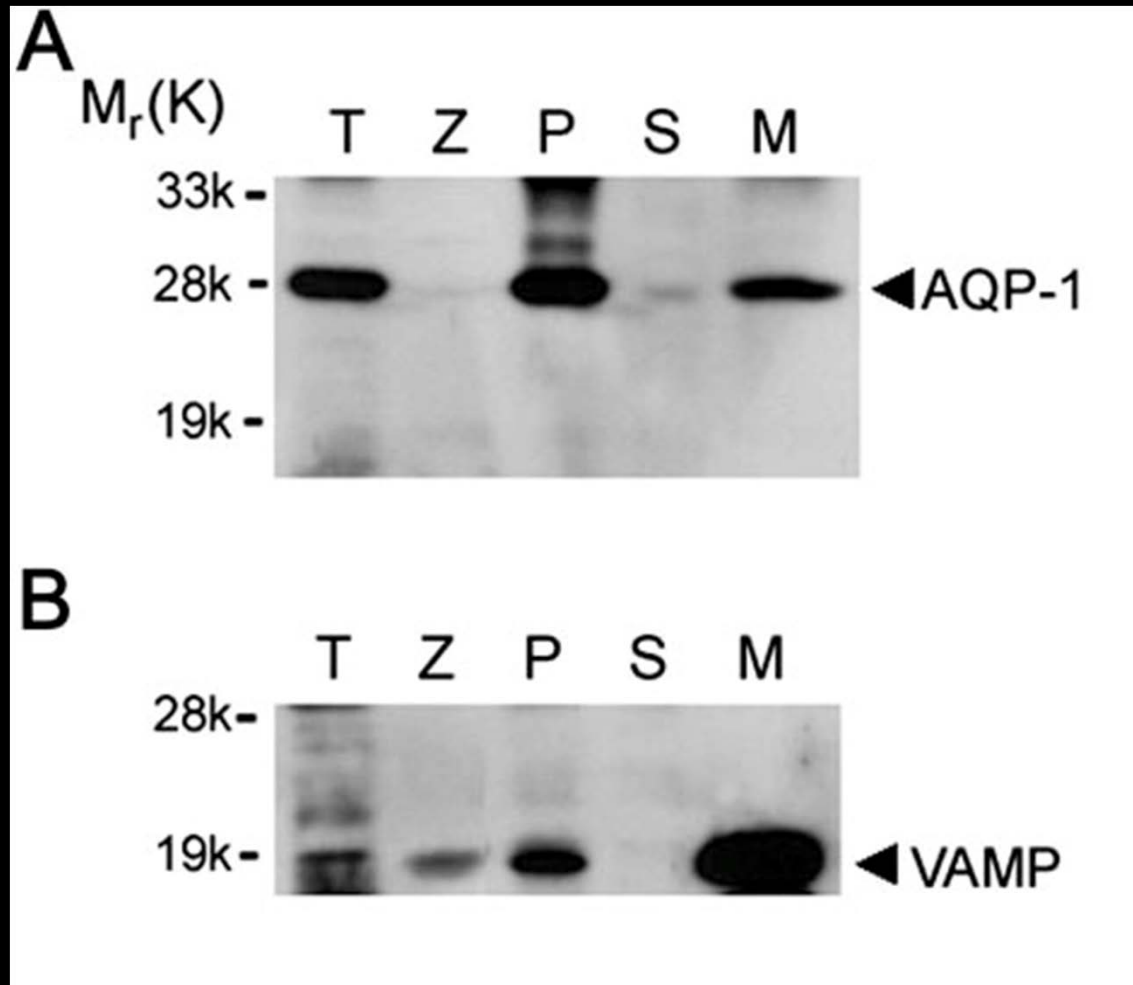


Kelly et. al. 2004 Cell Biol. Int. 28:709-16

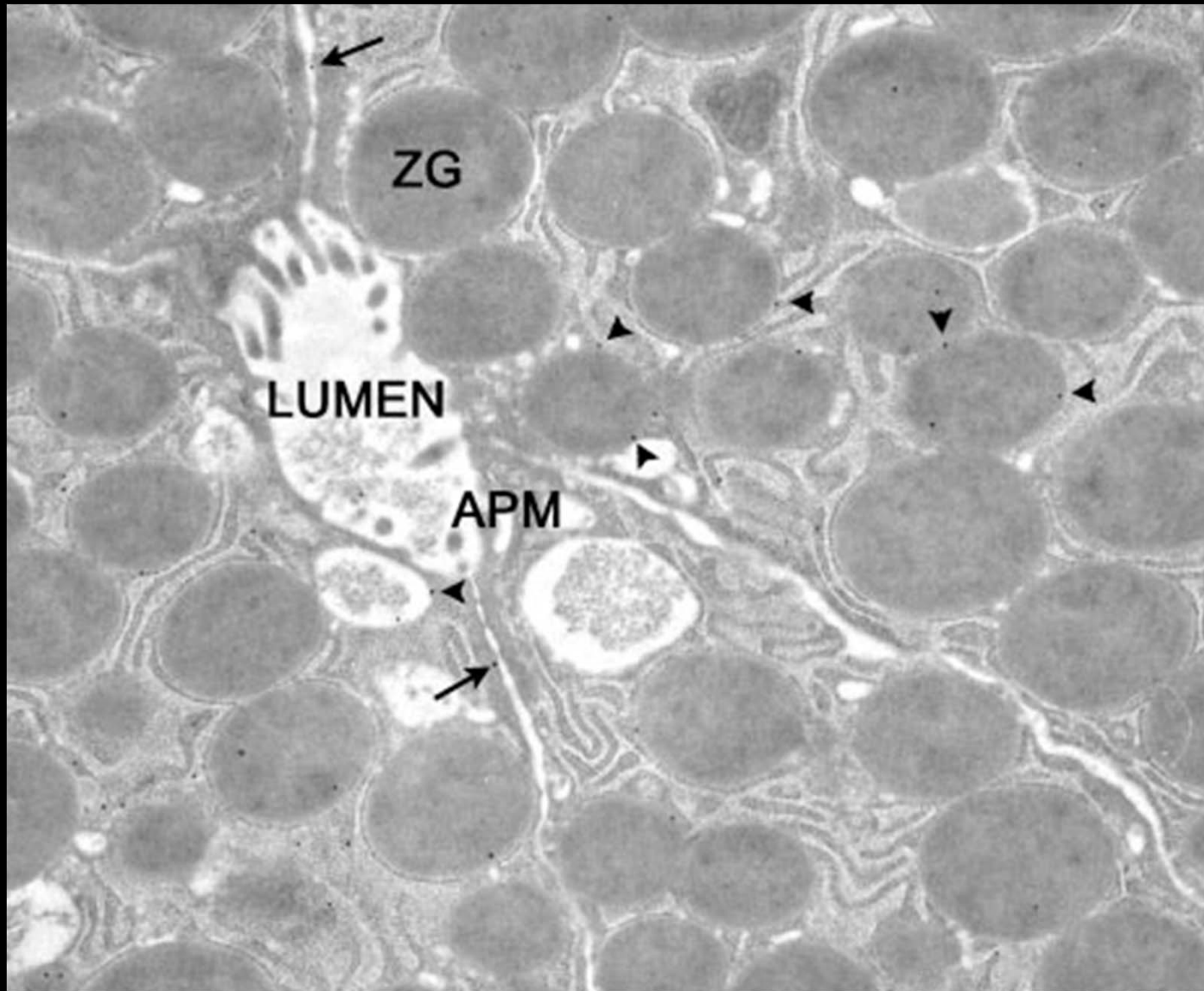


Jena et. al. 1997 Proc. Natl. Acad. Sci. USA 94:13317-22

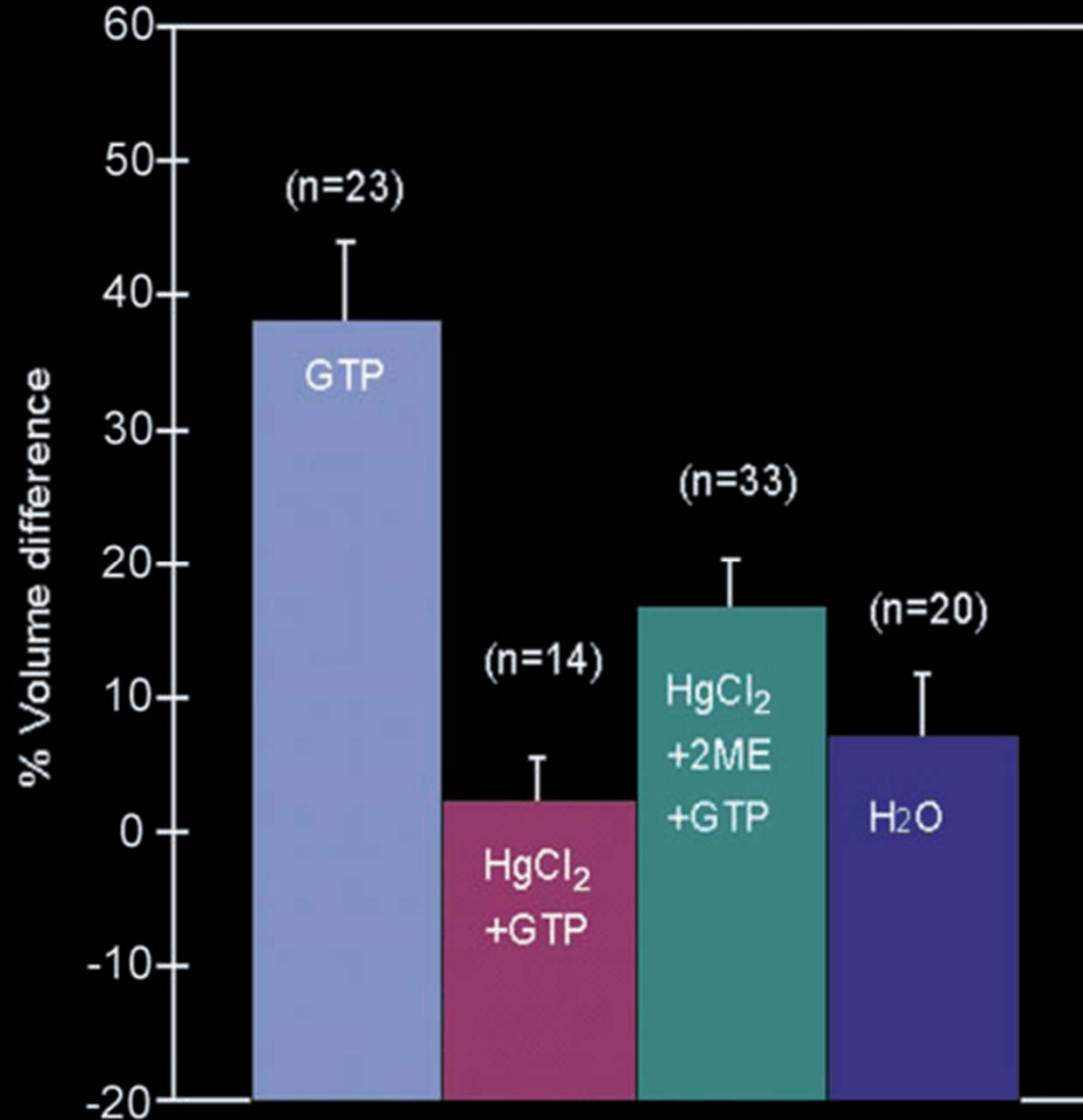
Water-Channel AQP1 at ZG Membrane



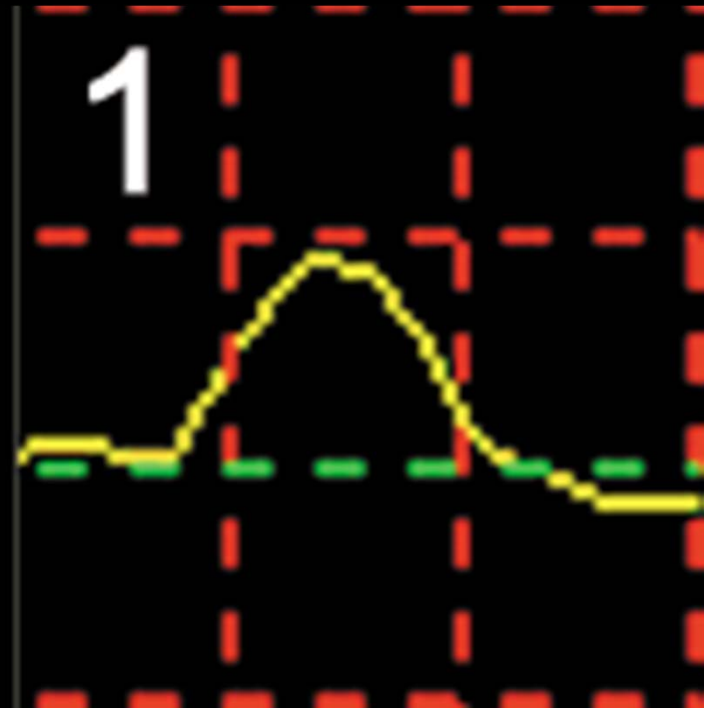
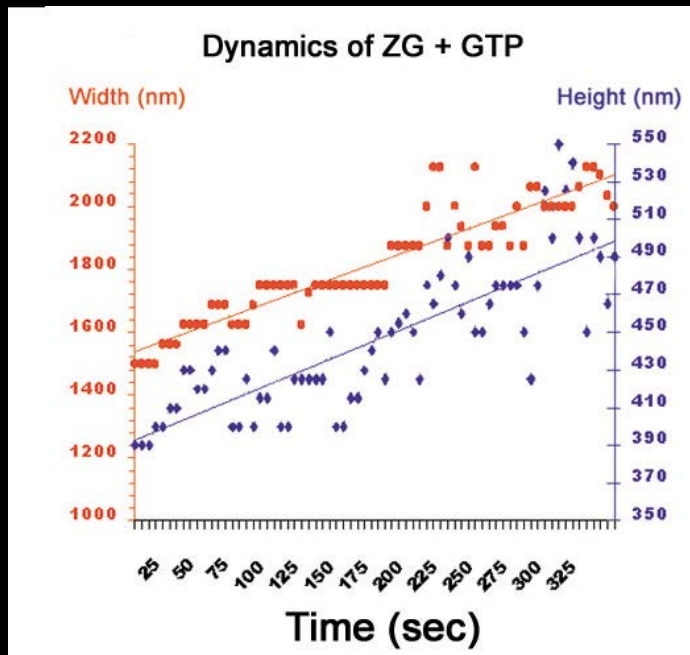
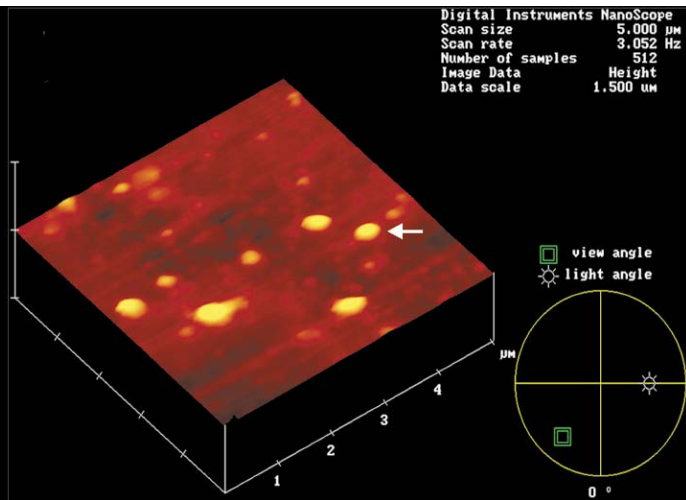
Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24



Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

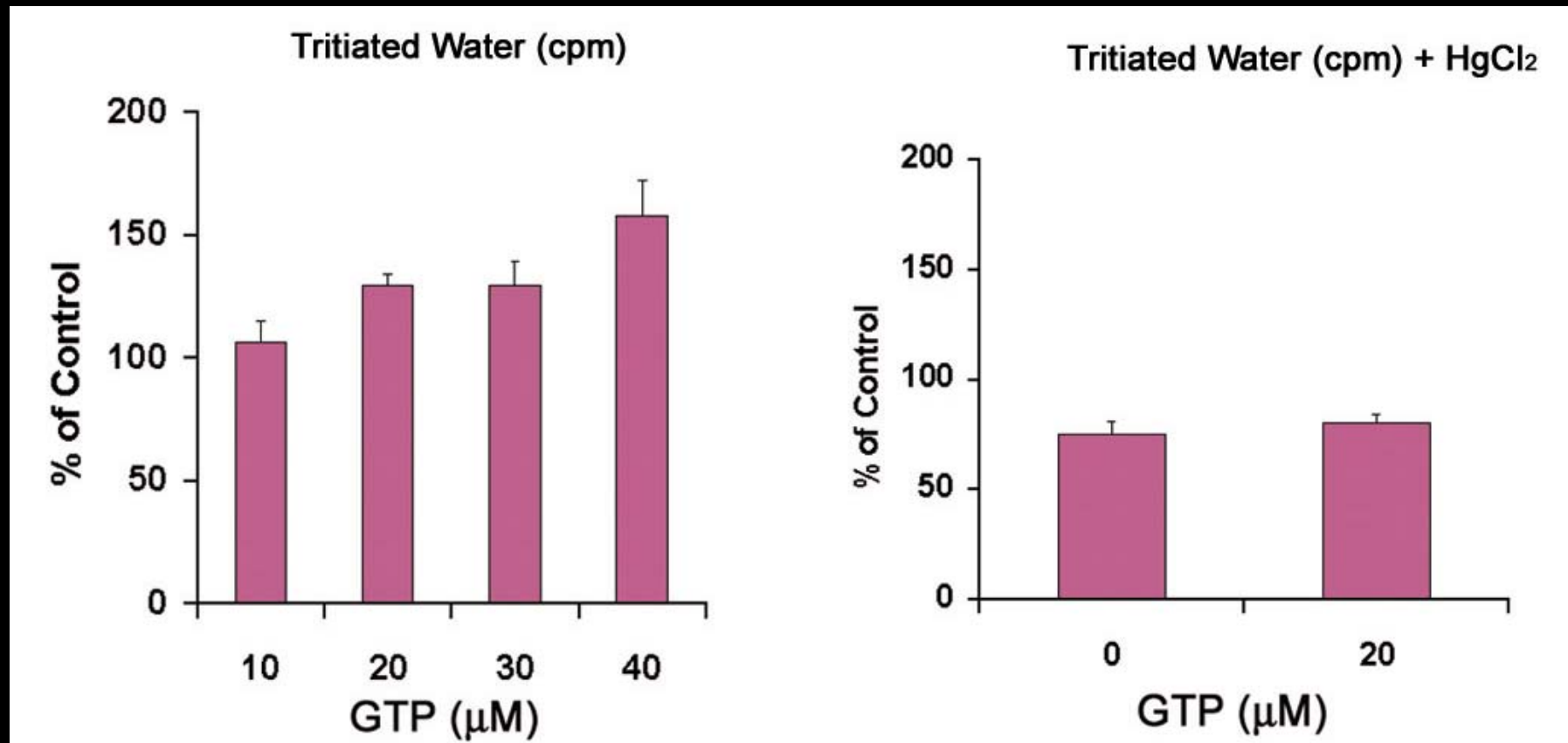


Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24



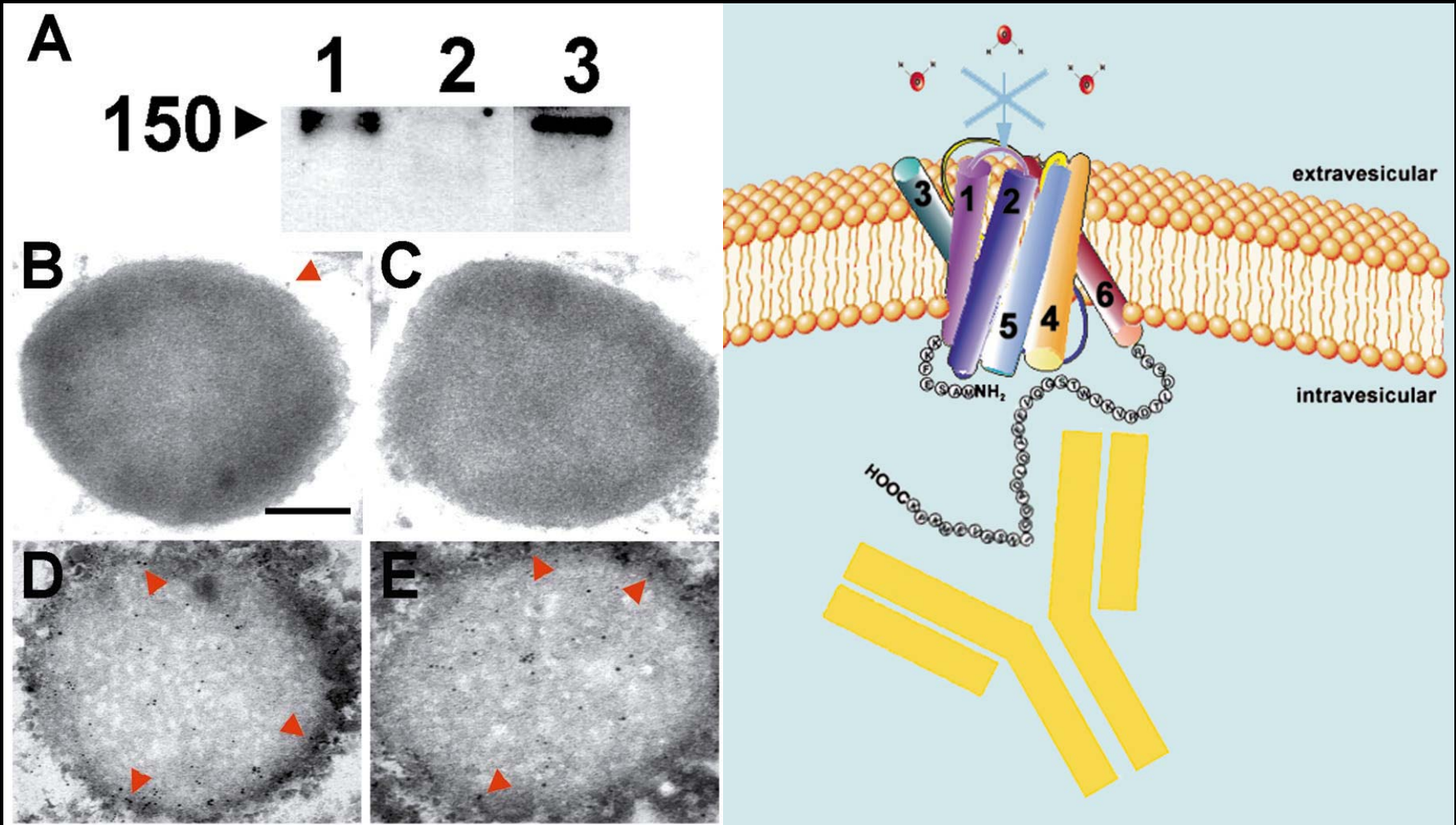
Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

Tritiated Water Entry into ZG

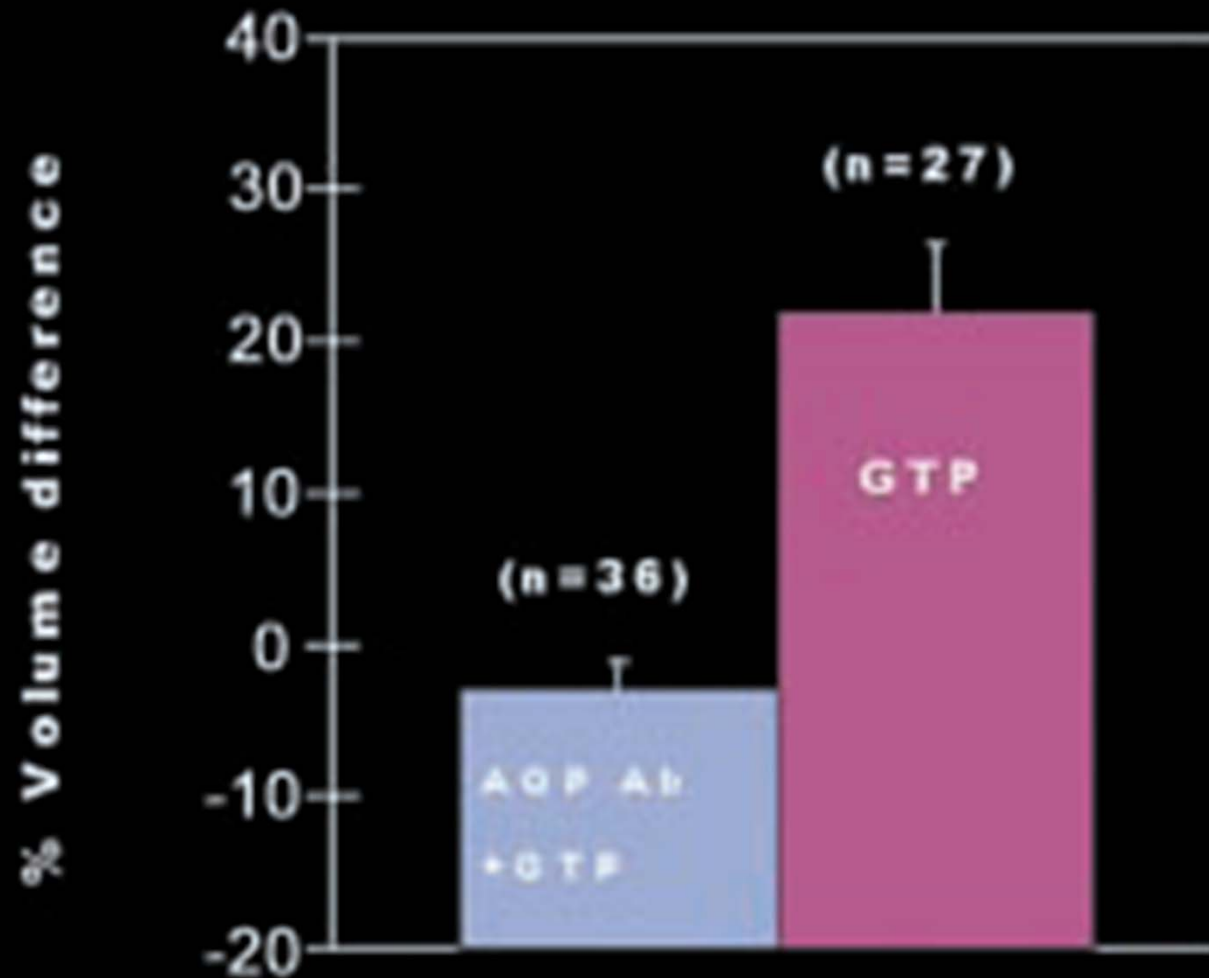


Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

Introduction of AQP1 antibody into ZG

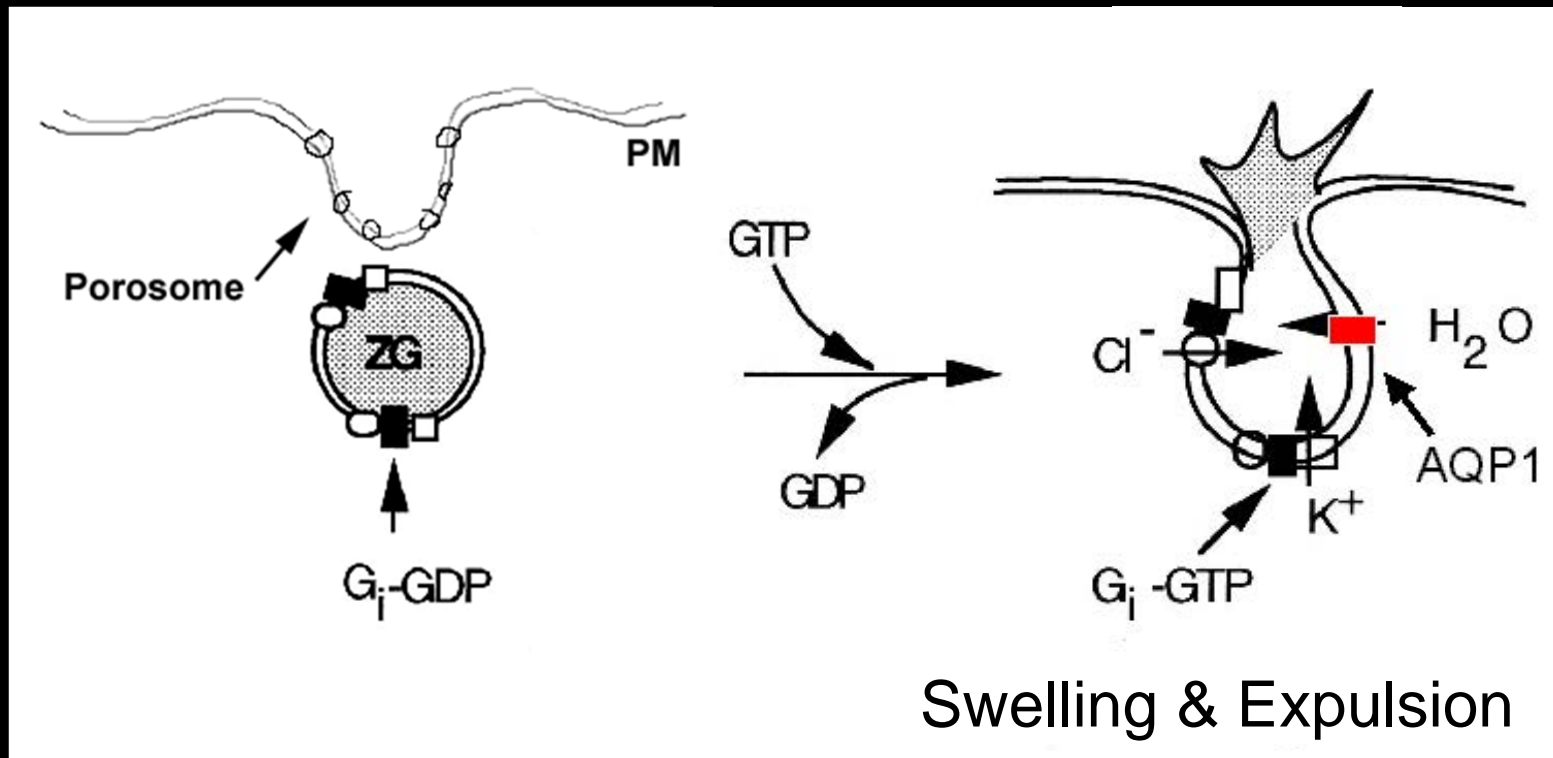


Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24



Cho et. al. 2002 Proc. Natl. Acad. Sci. USA 99:4720-24

Regulation of water entry into ZG

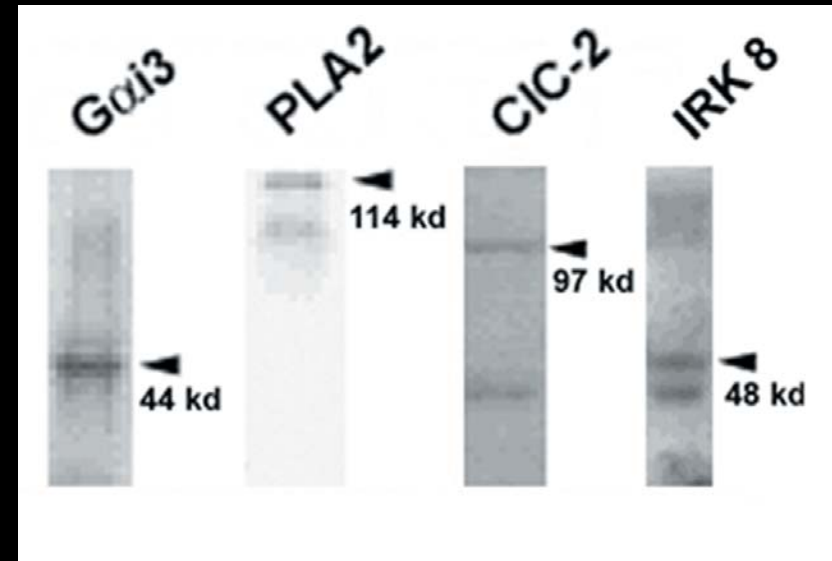


Jena et. al. 1997 PNAS Vol 94. 13317-22

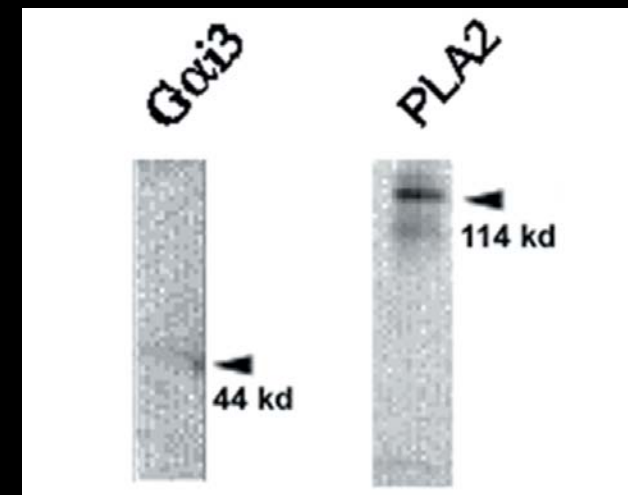
Cho et. al. 2002 PNAS Vol 99. 4720-24

AQP1 Immunoisolated Complex

Zymogene granules

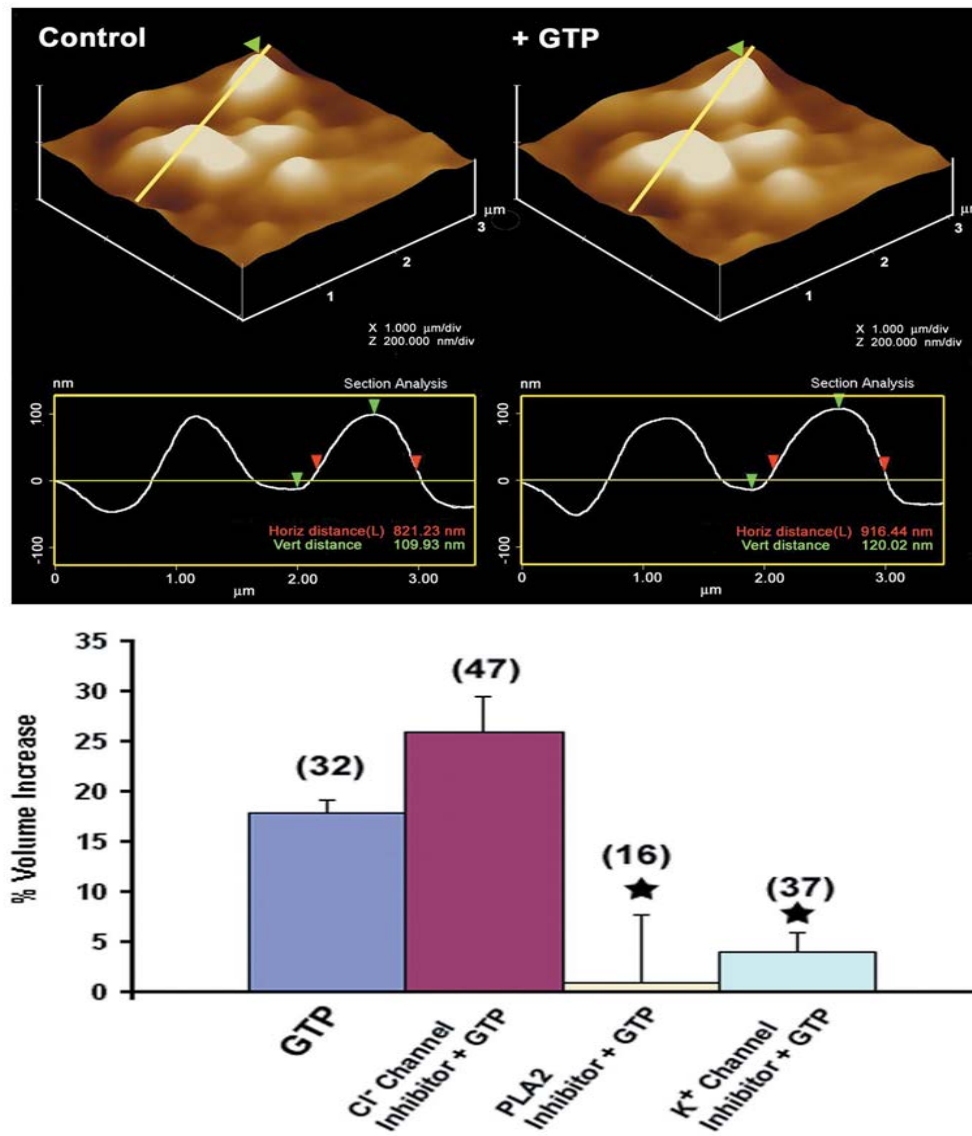


Red blood cells



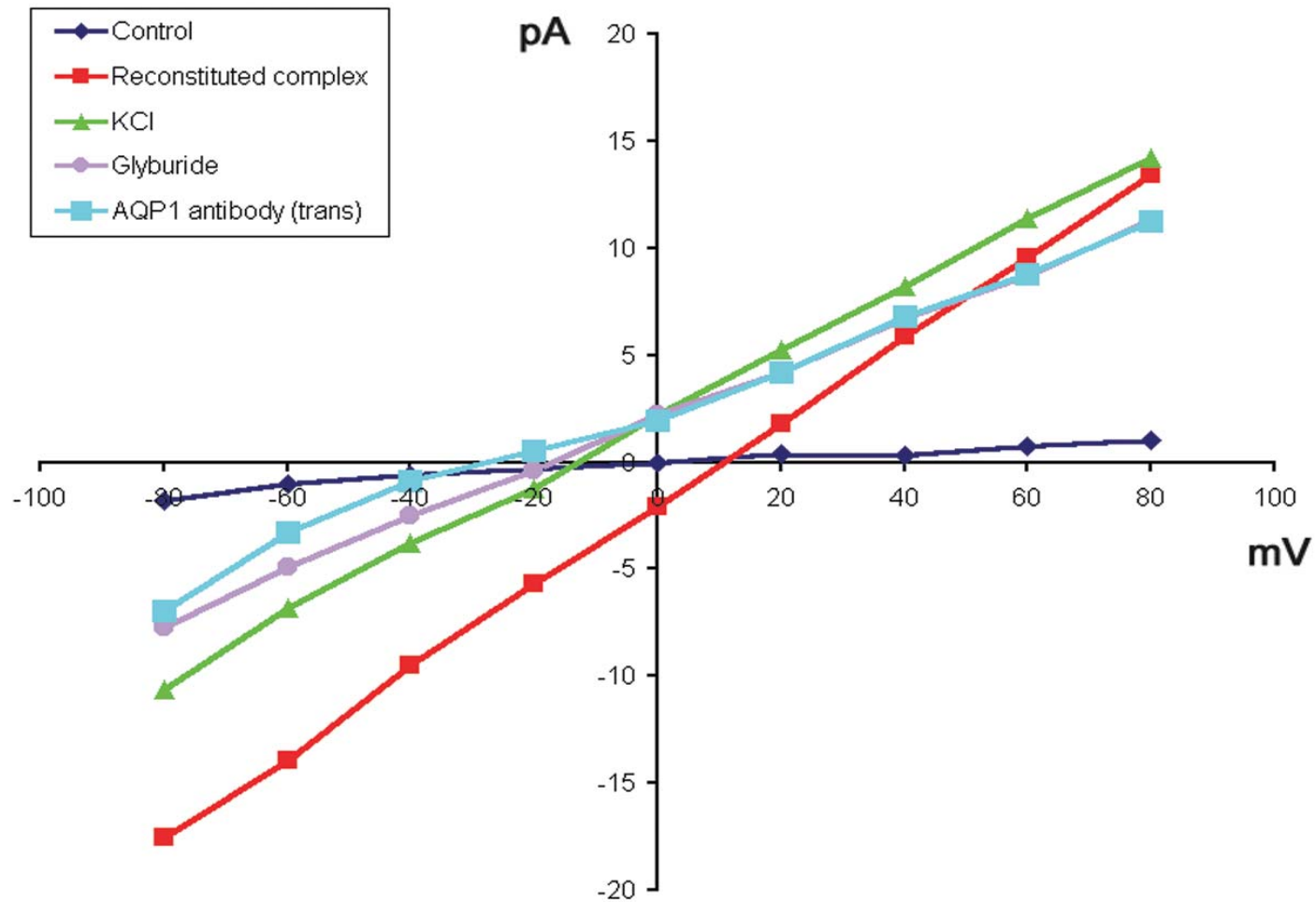
Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17

ZG volume changes measured by AFM



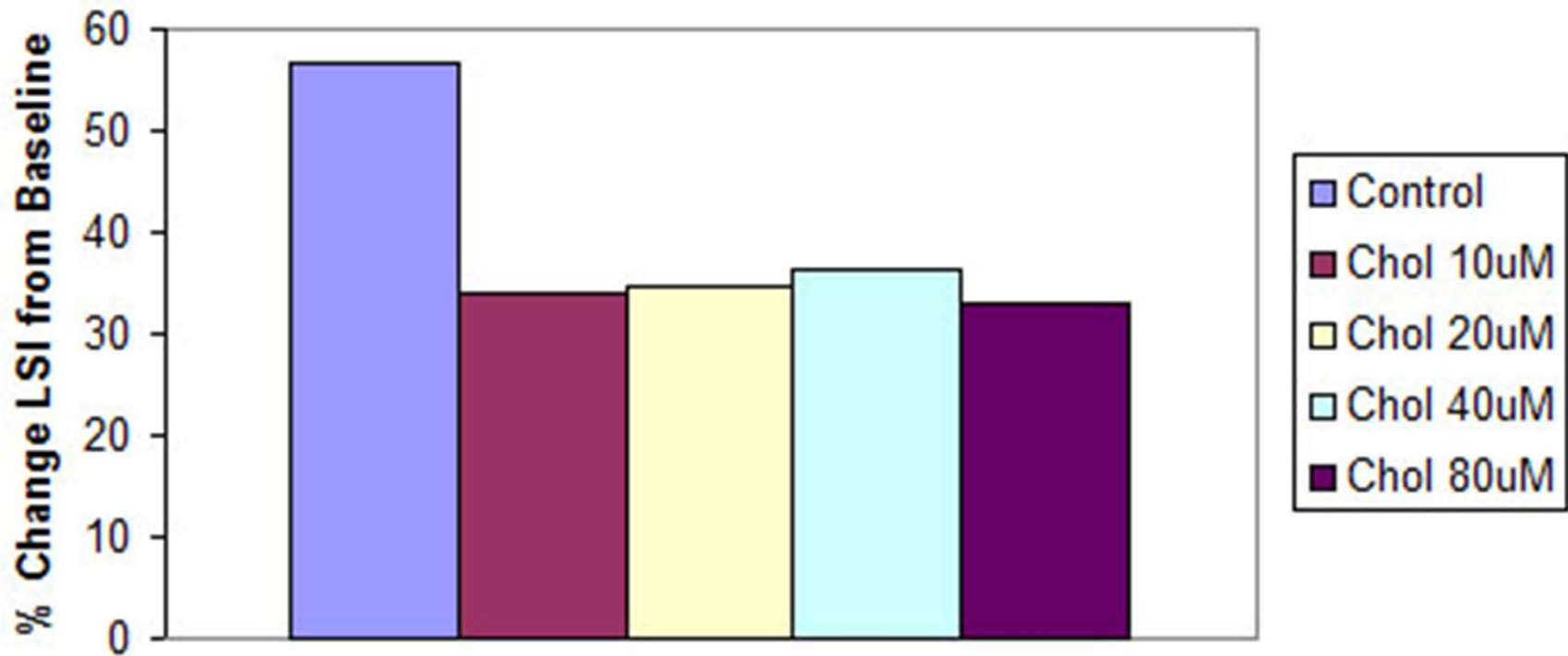
Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17

Electrophysiological properties of AQP1-immunocomplex reconstituted in PC:PS bilayer

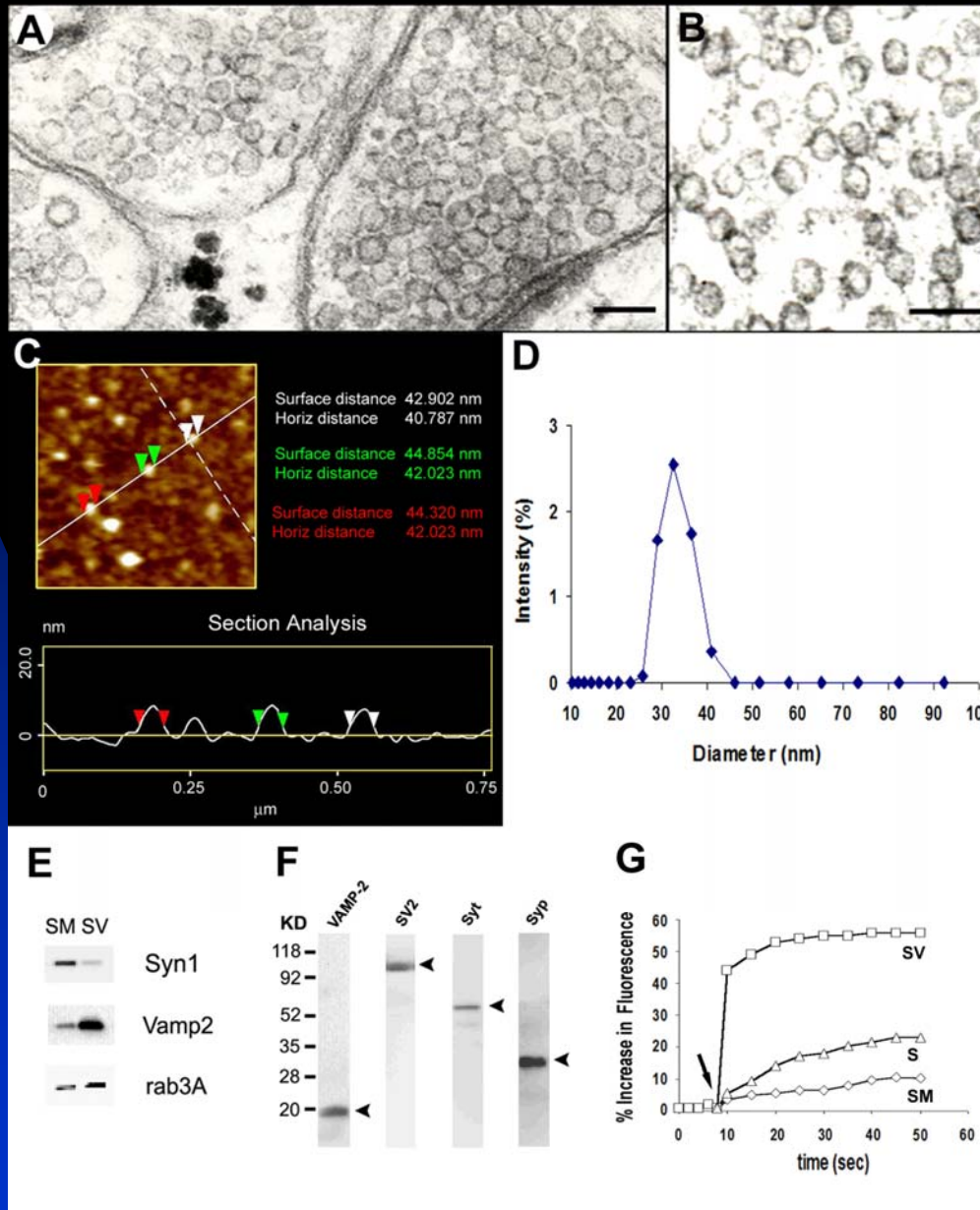


Abu-Hamdah et. al. 2004 Cell Biol. Int. 28:7-17

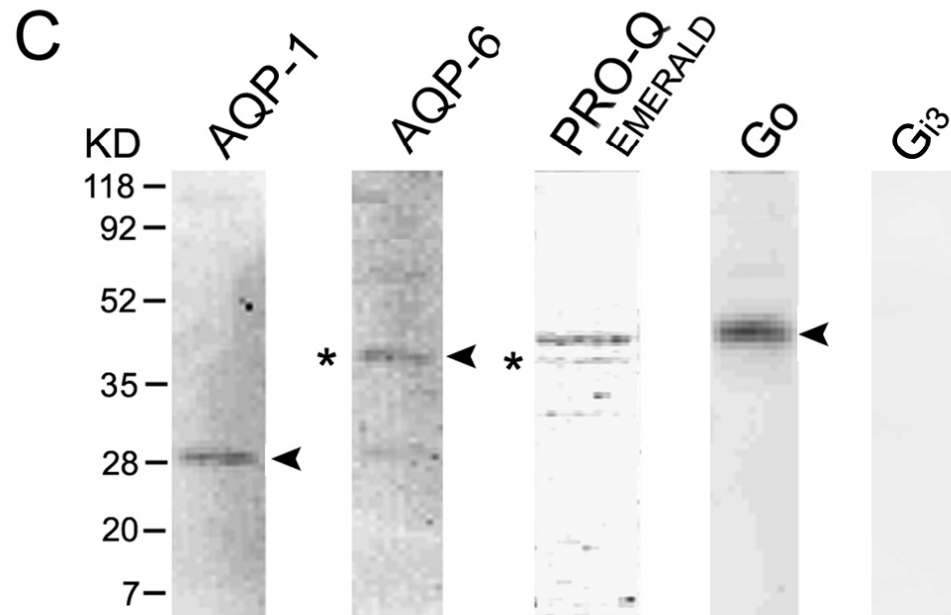
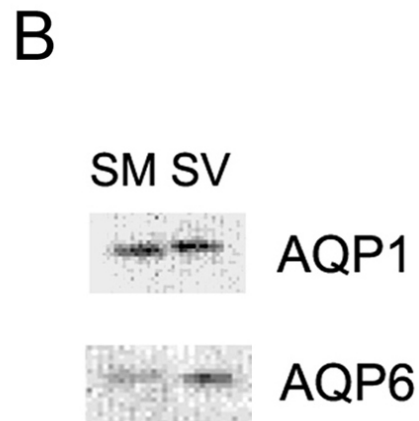
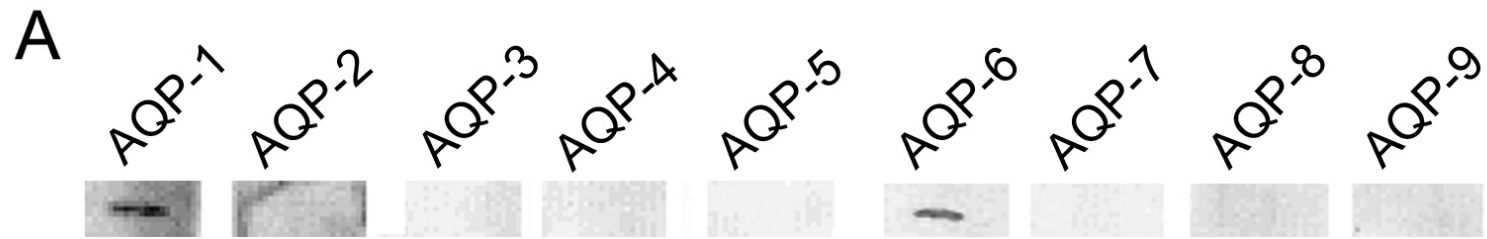
ZG Swelling after Cholesterol Incubation



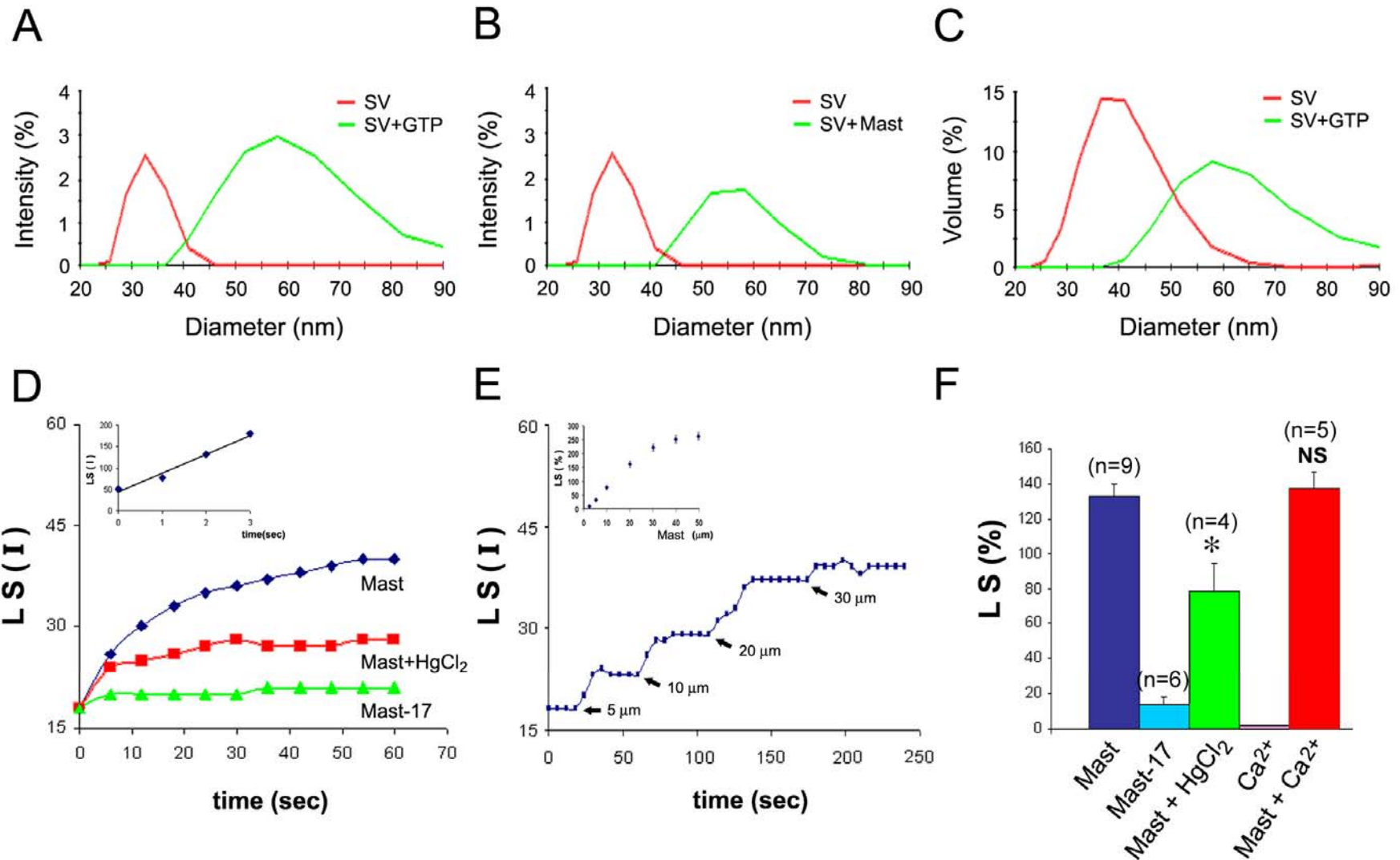
Amanda F., Jena B.P. (unpublished observation)



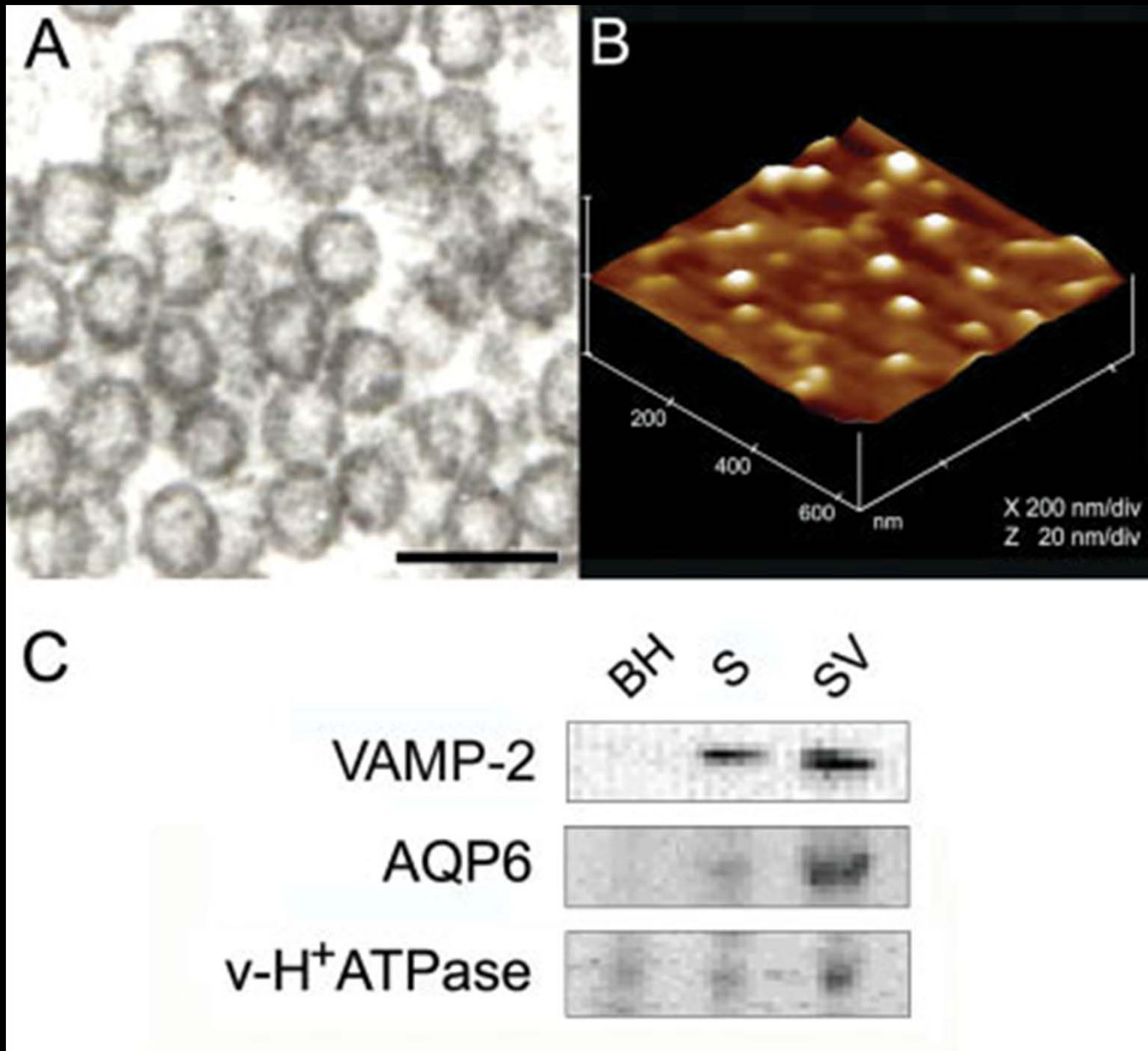
Jeremic et. al. 2005 Exp. Biol. Med. 230:674-80



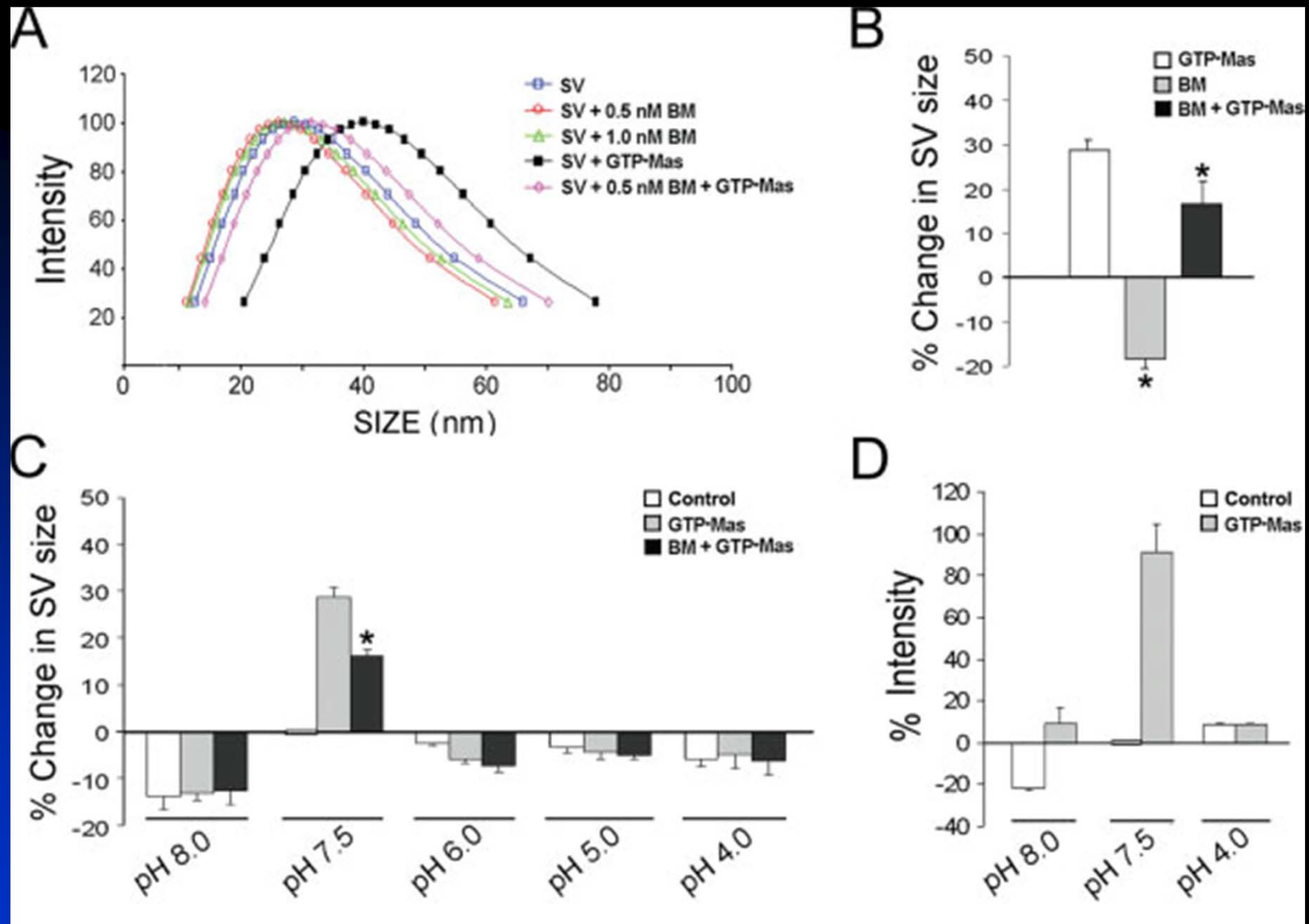
Jeremic et. al. 2005 Exp. Biol. Med. 230:674-80



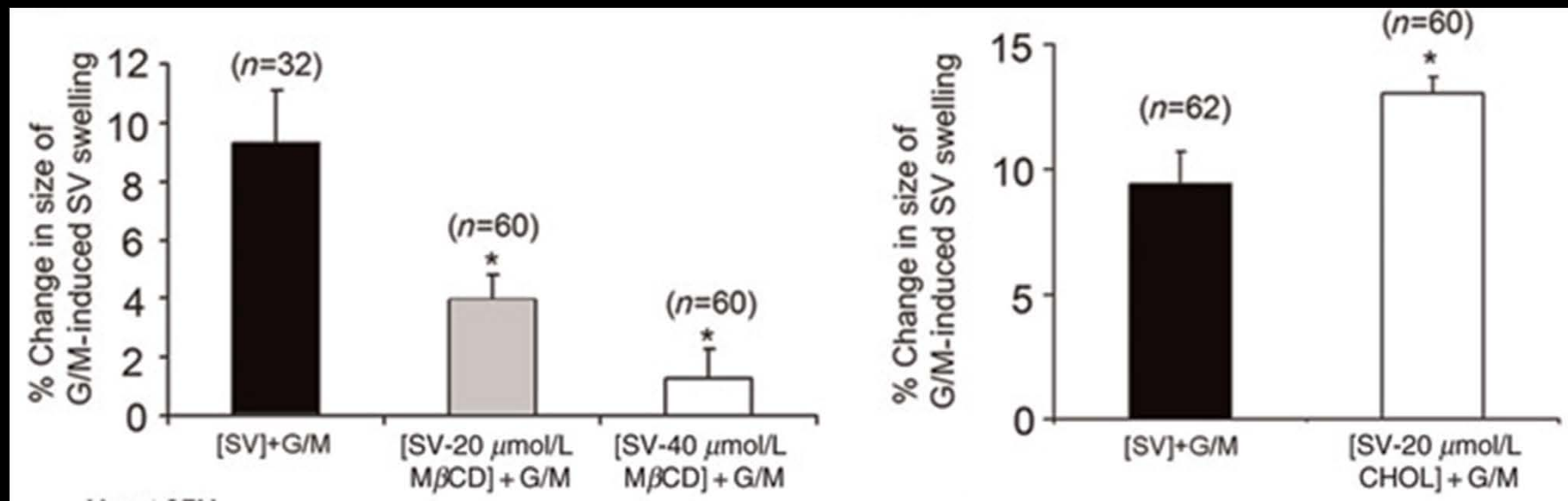
Jeremic et. al. 2005 Exp. Biol. Med. 230:674-80



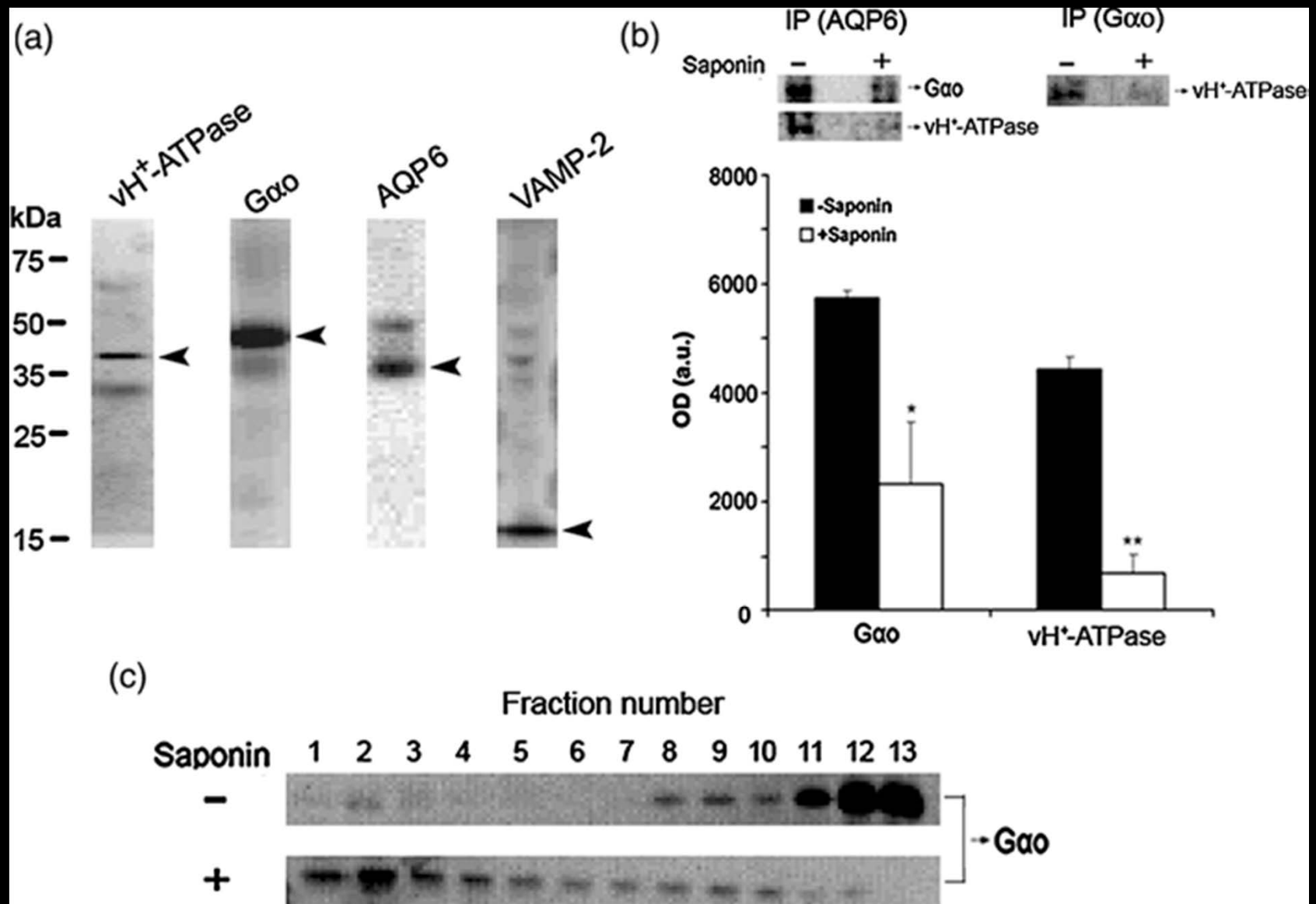
Shin et. al. 2010 J. Neurosci. Res. 88:95-101



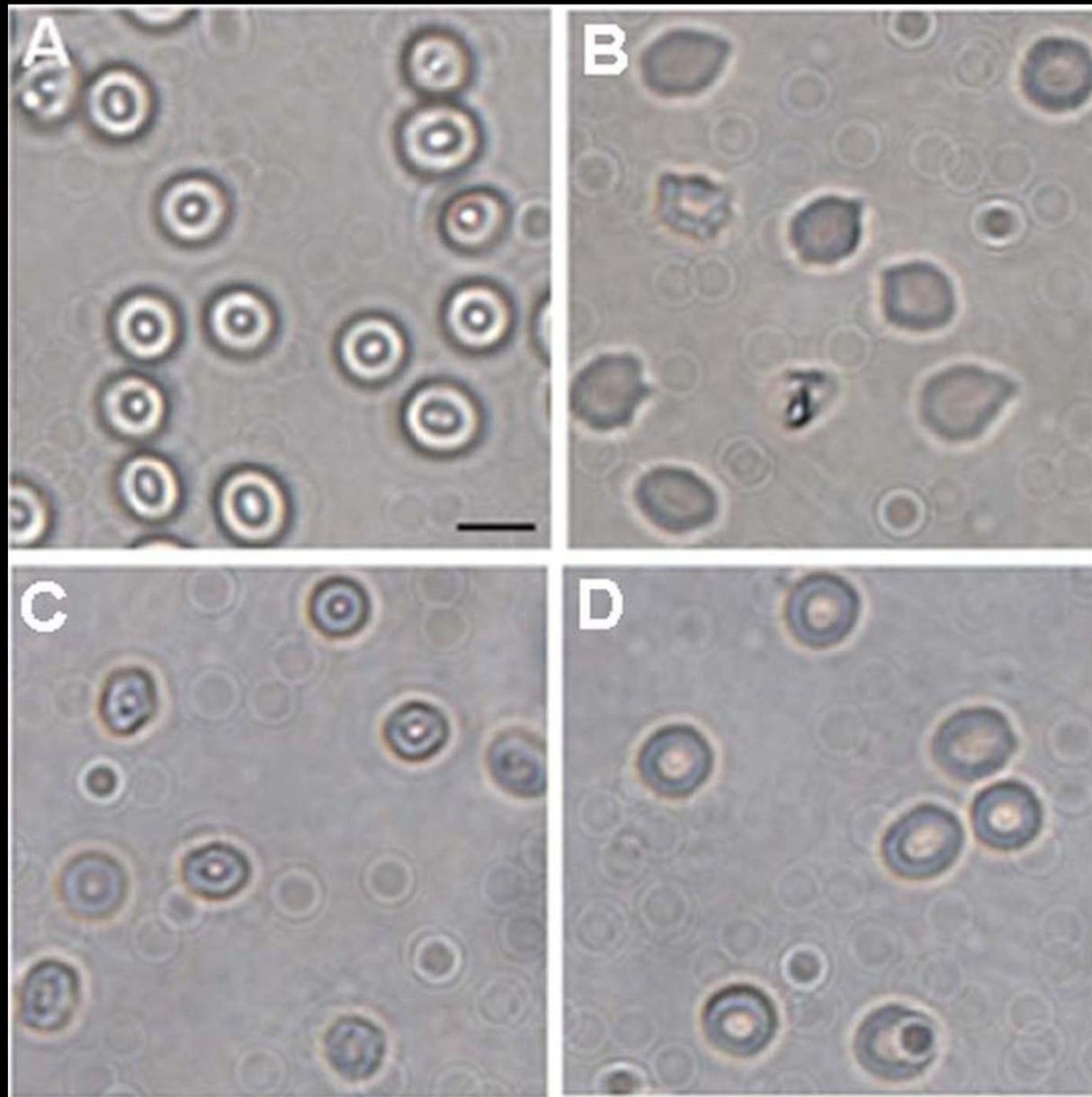
Shin et. al. 2010 J. Neurosci. Res. 88:95-101



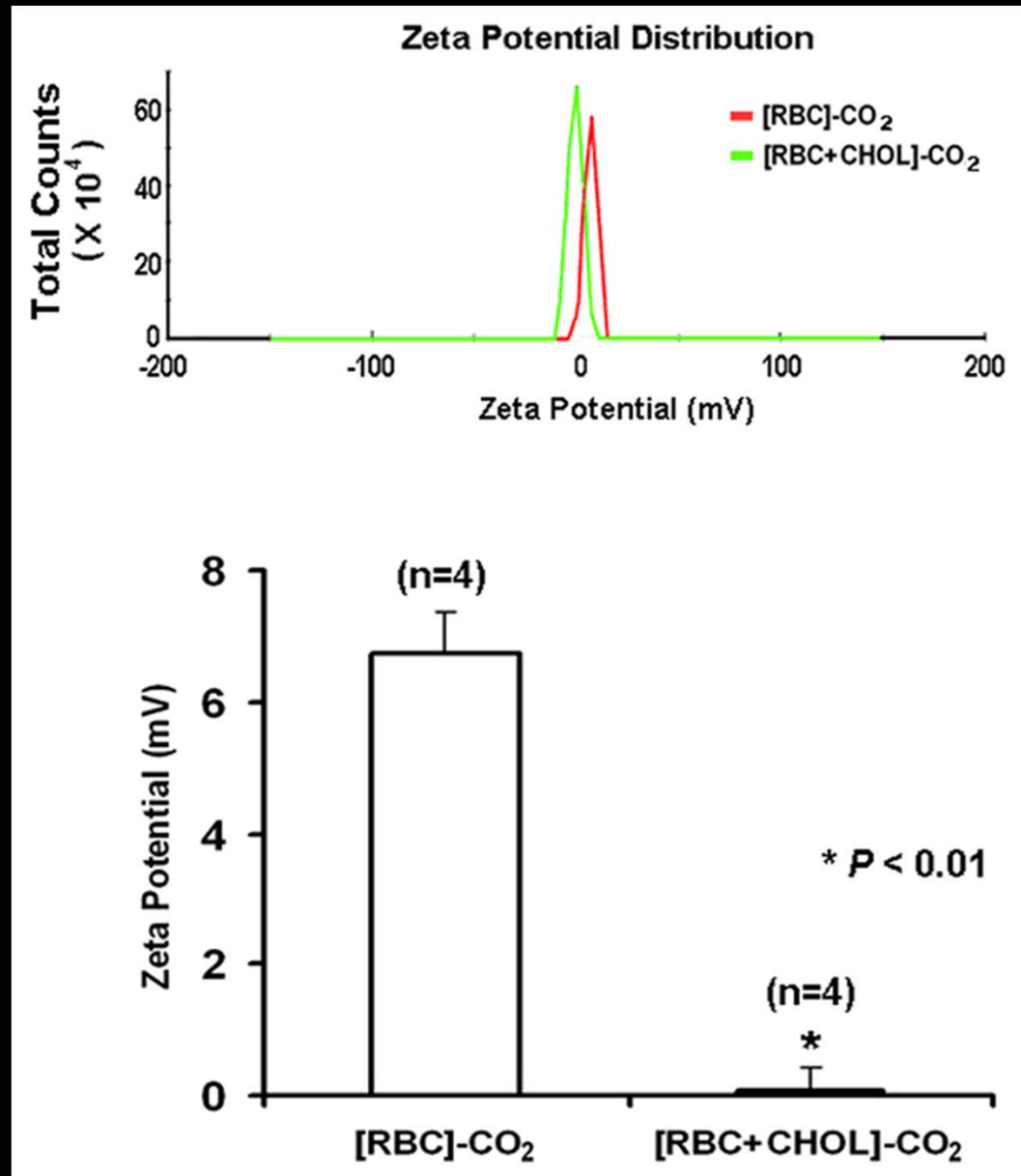
Lee et. al. 2010 Exp. Biol. Med. 235:470-7



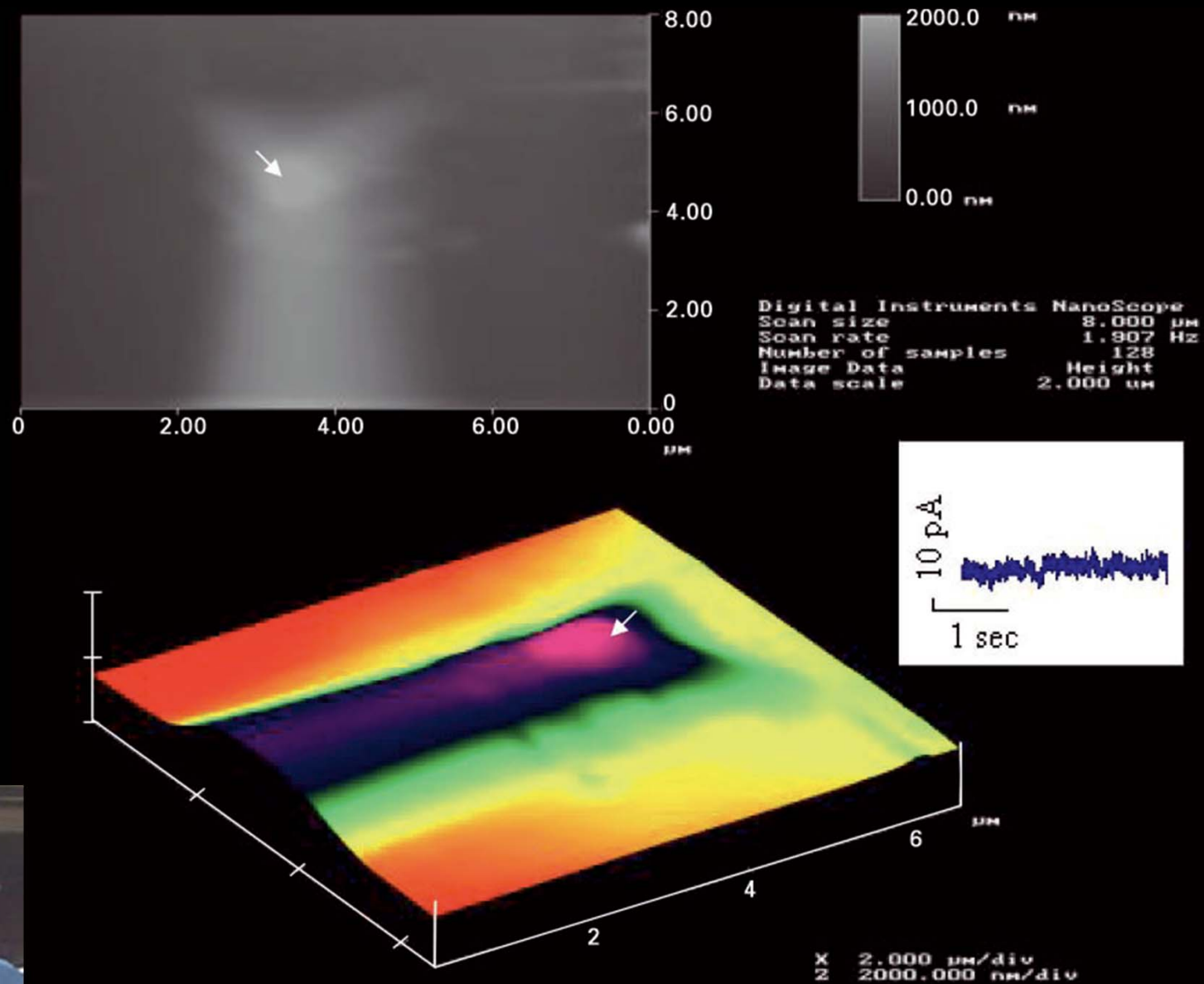
Lee et. al. 2010 Exp. Biol. Med. 235:470-7



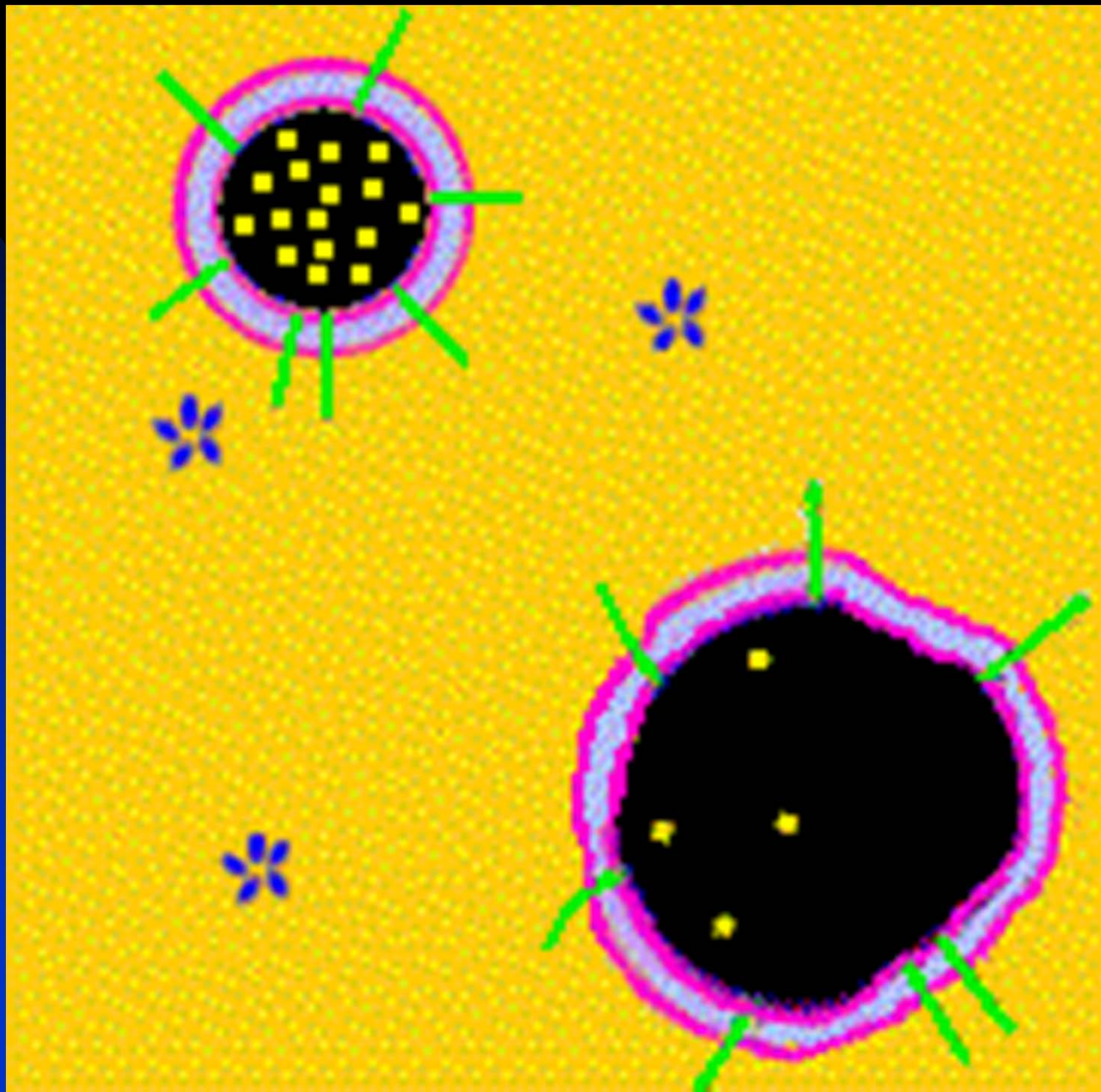
Jena S.G., Lee J.-S. 2010 J. Biol. Phys. Chem. 10:127-34



Jena S.G., Lee J.-S. 2010 J. Biol. Phys. Chem. 10:127-34



Kelly et al., 2005 Pancreatology 5:443-49

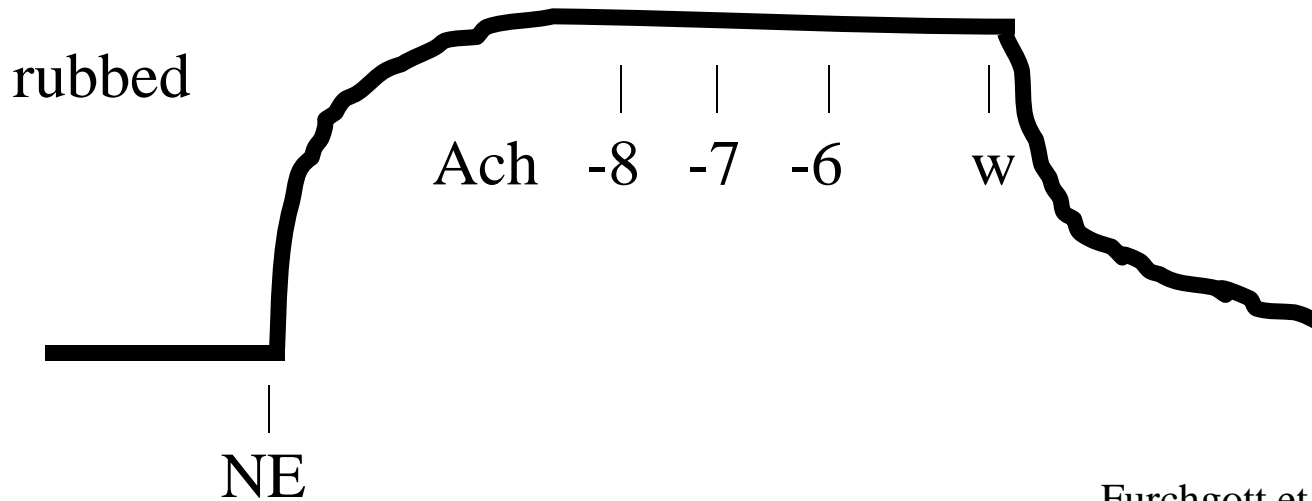
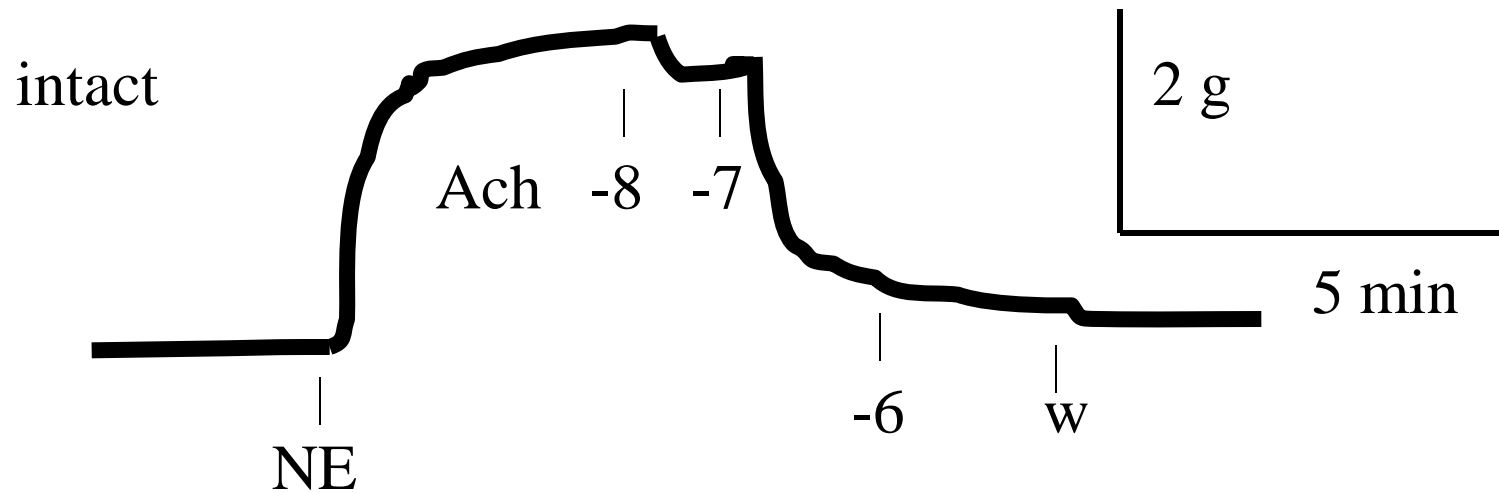


The truth about the movement of NO across cell membranes

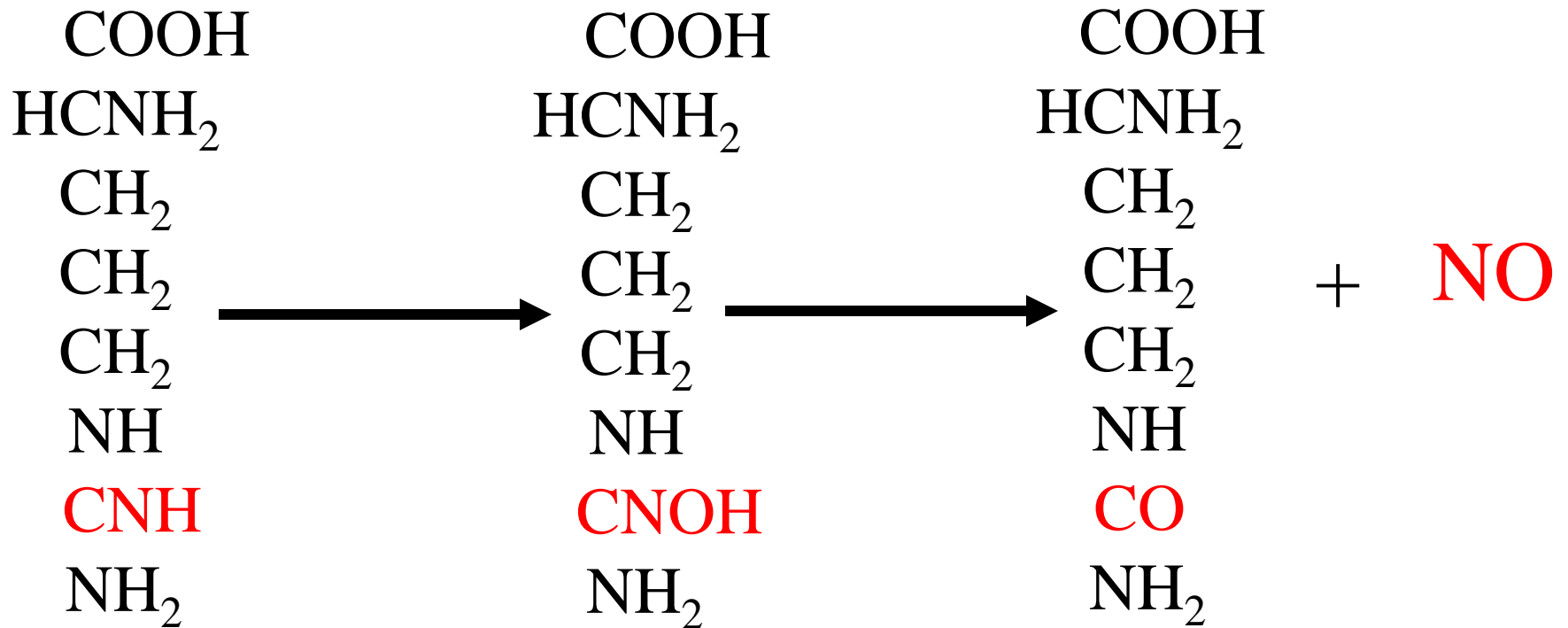
Jeffrey Garvin

Hypertension and Vascular Research Division
Department of Internal Medicine
Henry Ford Hospital

Acetylcholine-induced EDRF release



NO synthesis



L-arginine

L-citrulline

Why do we care about NO?

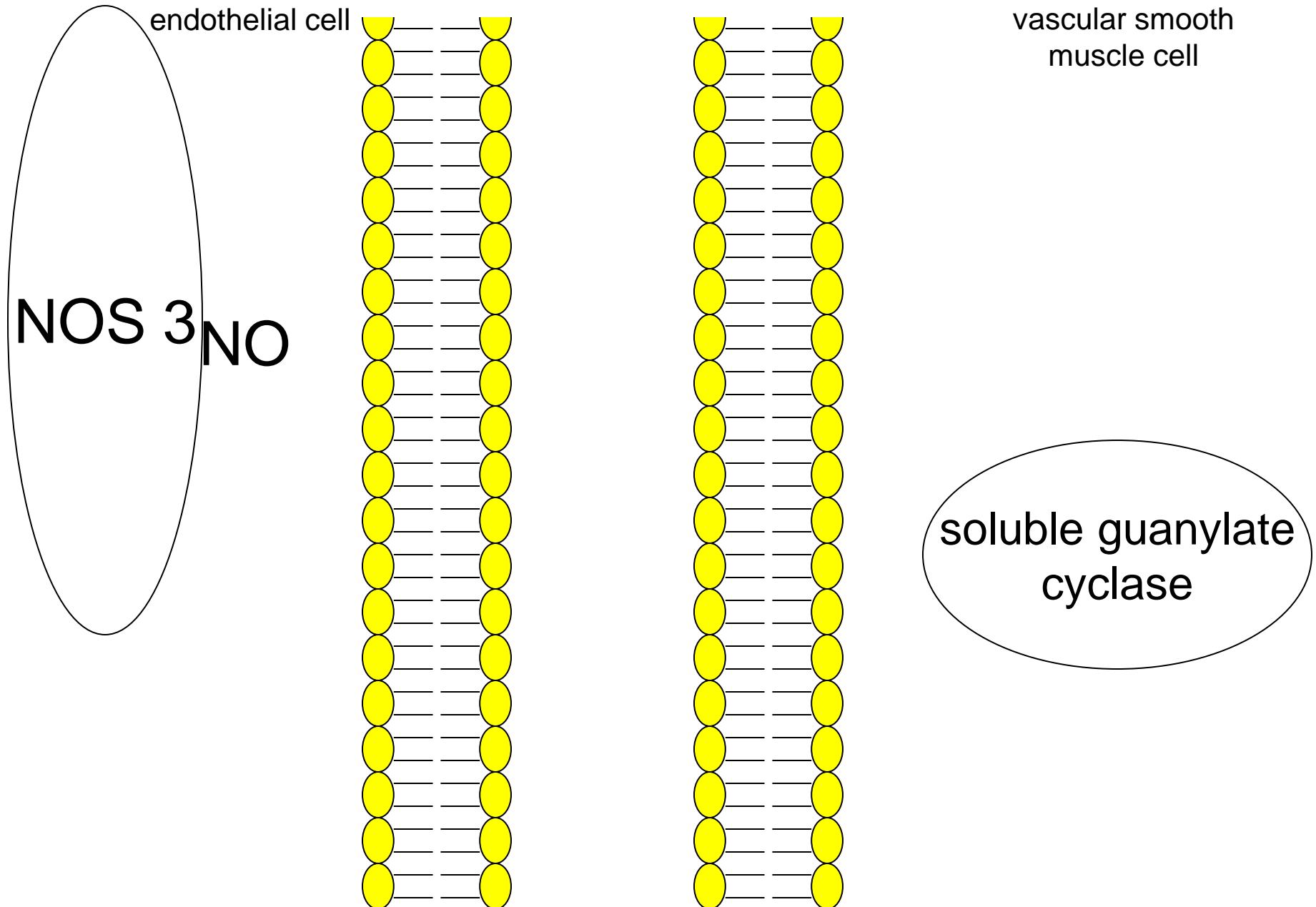
NO is involved in:

1. CNS function and cognition
2. Cardiac contractility
3. Peripheral vascular resistance
4. Respiration
5. Gut motility and ion absorption
6. Renal perfusion and transport
7. Reproduction

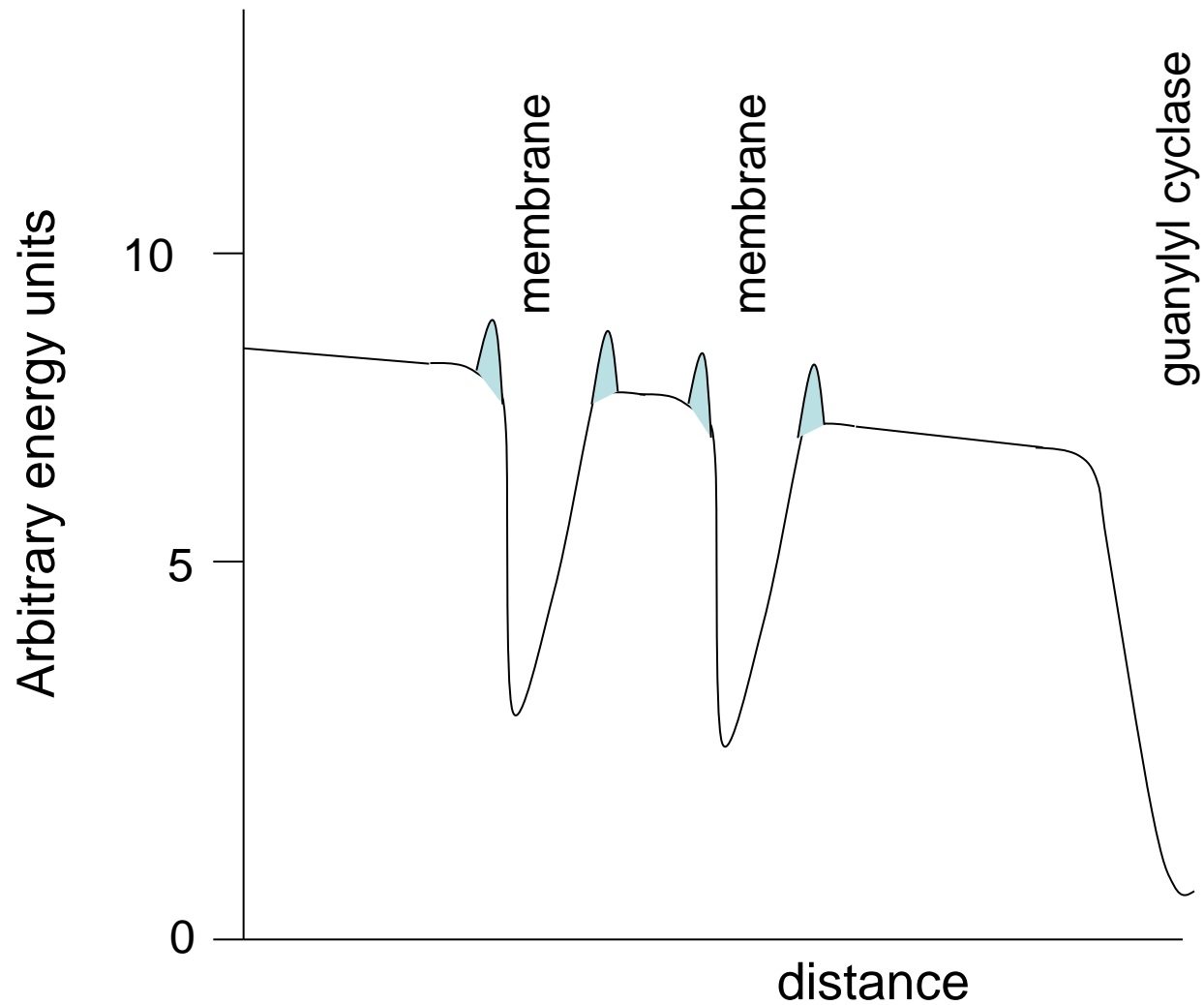
Properties of NO

1. It is small.
2. It is non-polar.
3. It is RELATIVELY lipophilic with a partition coefficient of about 5.
4. It is a gas.
5. Its reactive (different from O₂ and CO₂).

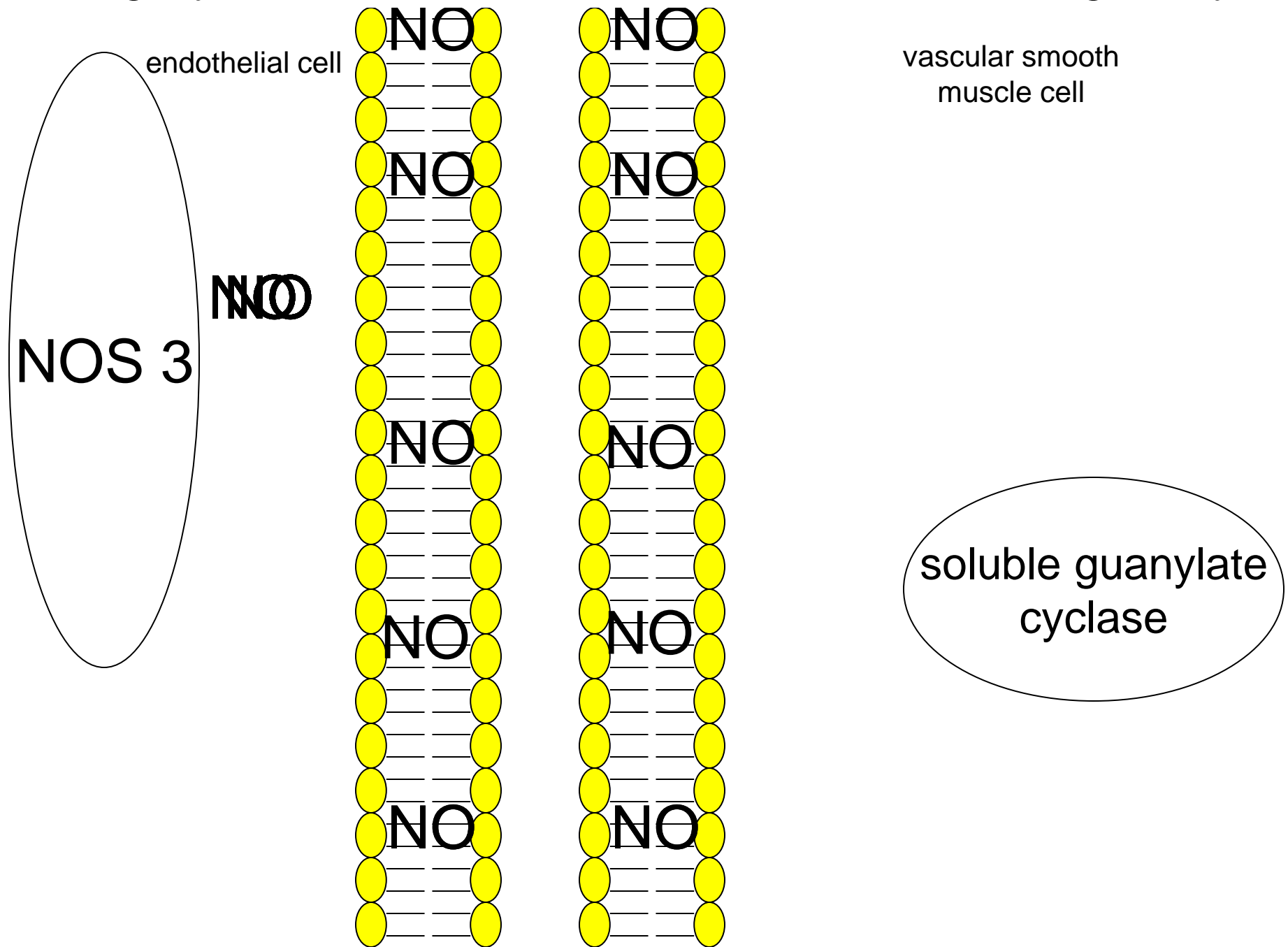
How many think NO diffuses through two bilayers



Energy profile of NO with distance based on partition coefficient



A slightly more “realistic” model of NO diffusion through bilayers



There have been no direct measurements of the NO permeability of any cell membrane!!!

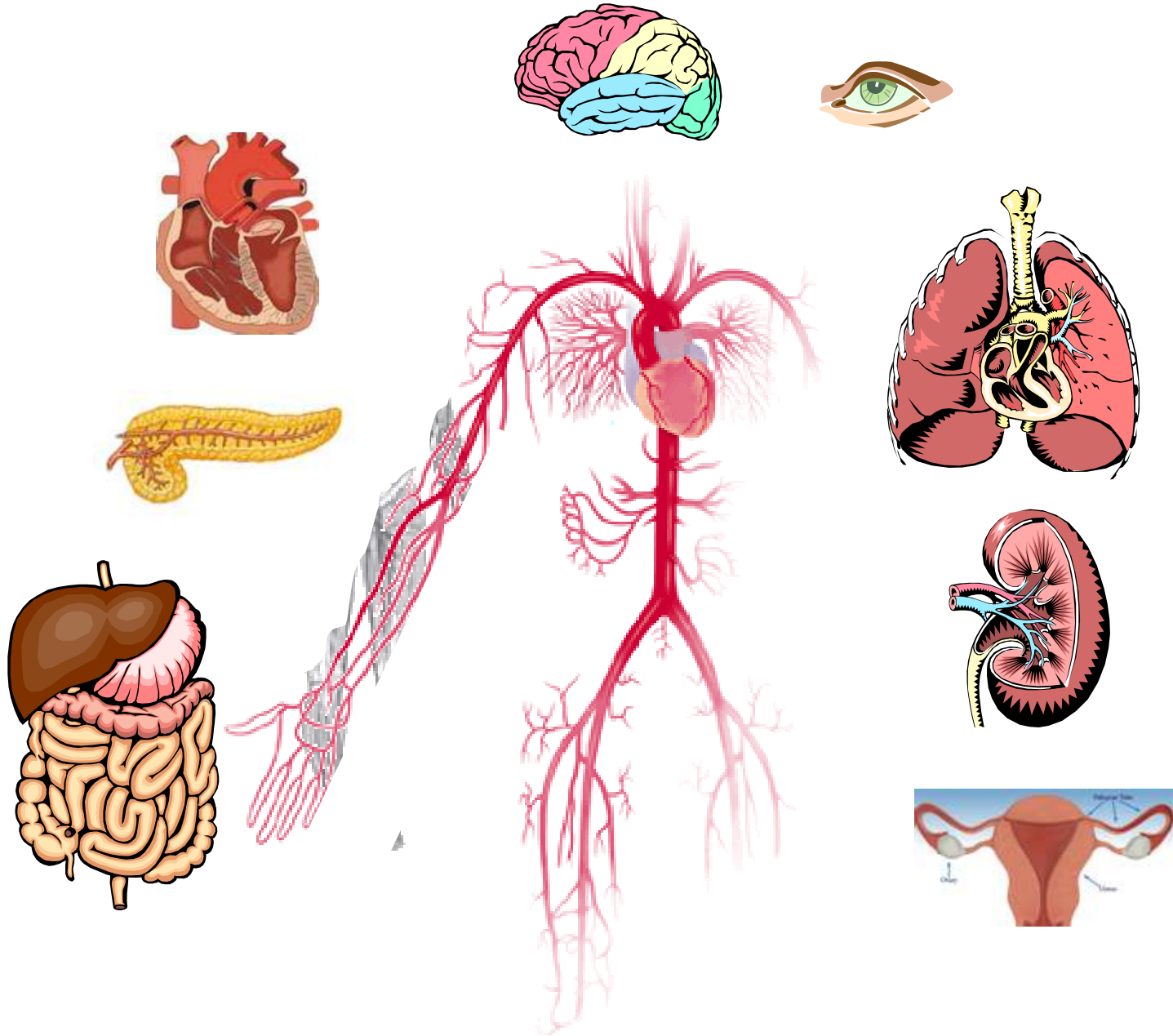
There has been one calculation which is widely cited. This value of 76 cm/s was calculated based on steady-state measurements of NO within an artificial membrane using 2 mM NO.

Free diffusion creates several problems:

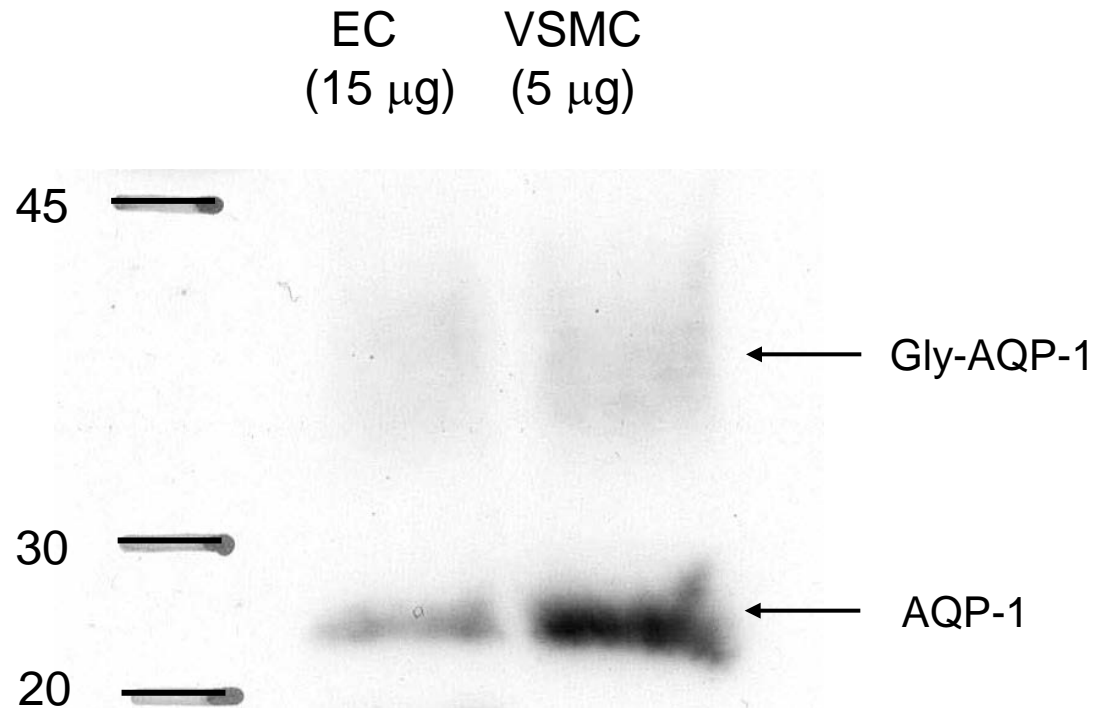
1. Free diffusion is relatively slow;
2. The amount of NO trapped in the membrane is relatively large;
3. If NO is only around transiently, the membrane could act as a trap;
4. There is no control over where NO goes;
5. There is no way to regulate NO release;
6. There is little control over NO entry.

As you have heard today “gas channels” including aquaporin-1 (AQP-1) has been shown to transport CO₂ and other gases.

Organs where AQP-1 and NO synthase are expressed



AQP-1 expression by aortic EC and VSMC isolated from CD1 mice



Hypothesis

endothelial cell

vascular smooth
muscle cell

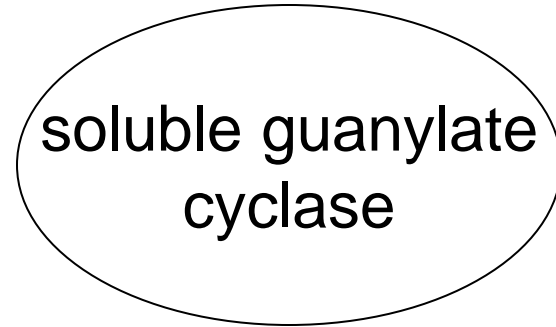
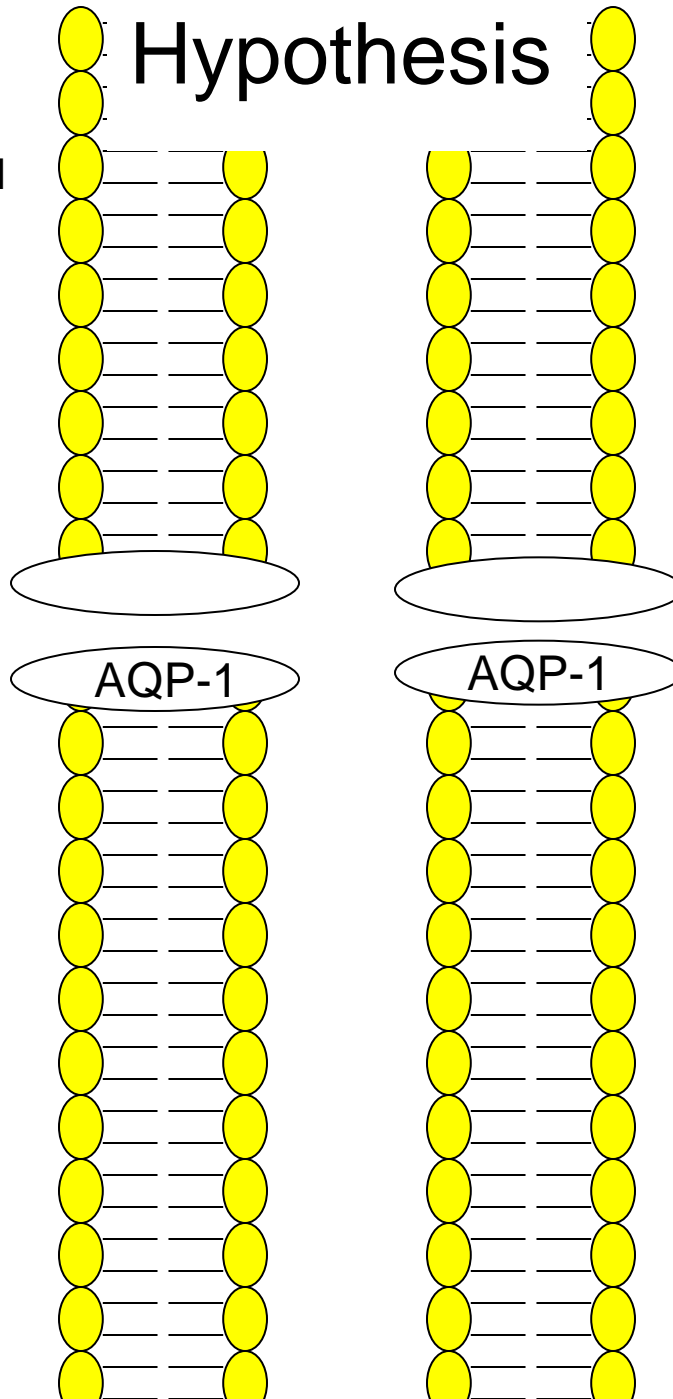
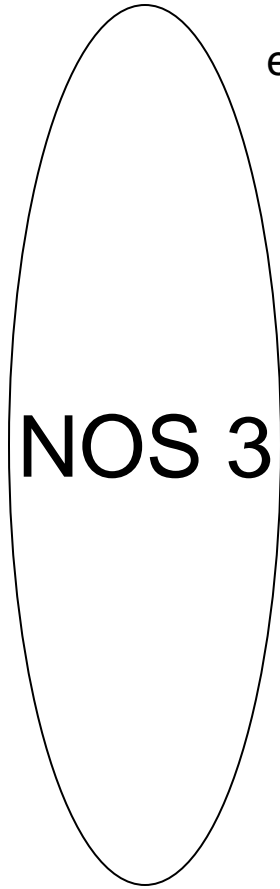
NOS 3

NO

AQP-1

AQP-1

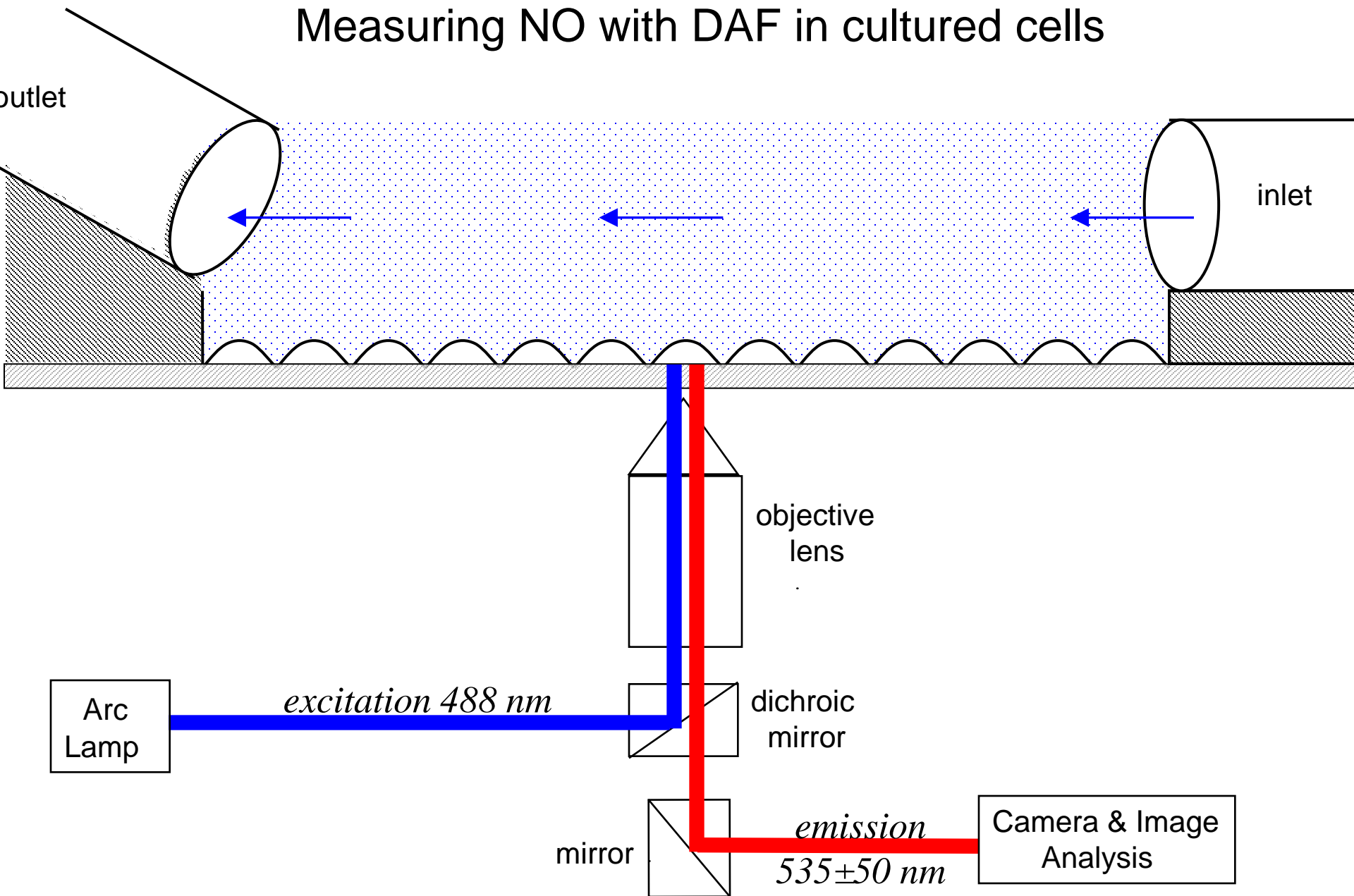
soluble guanylate
cyclase



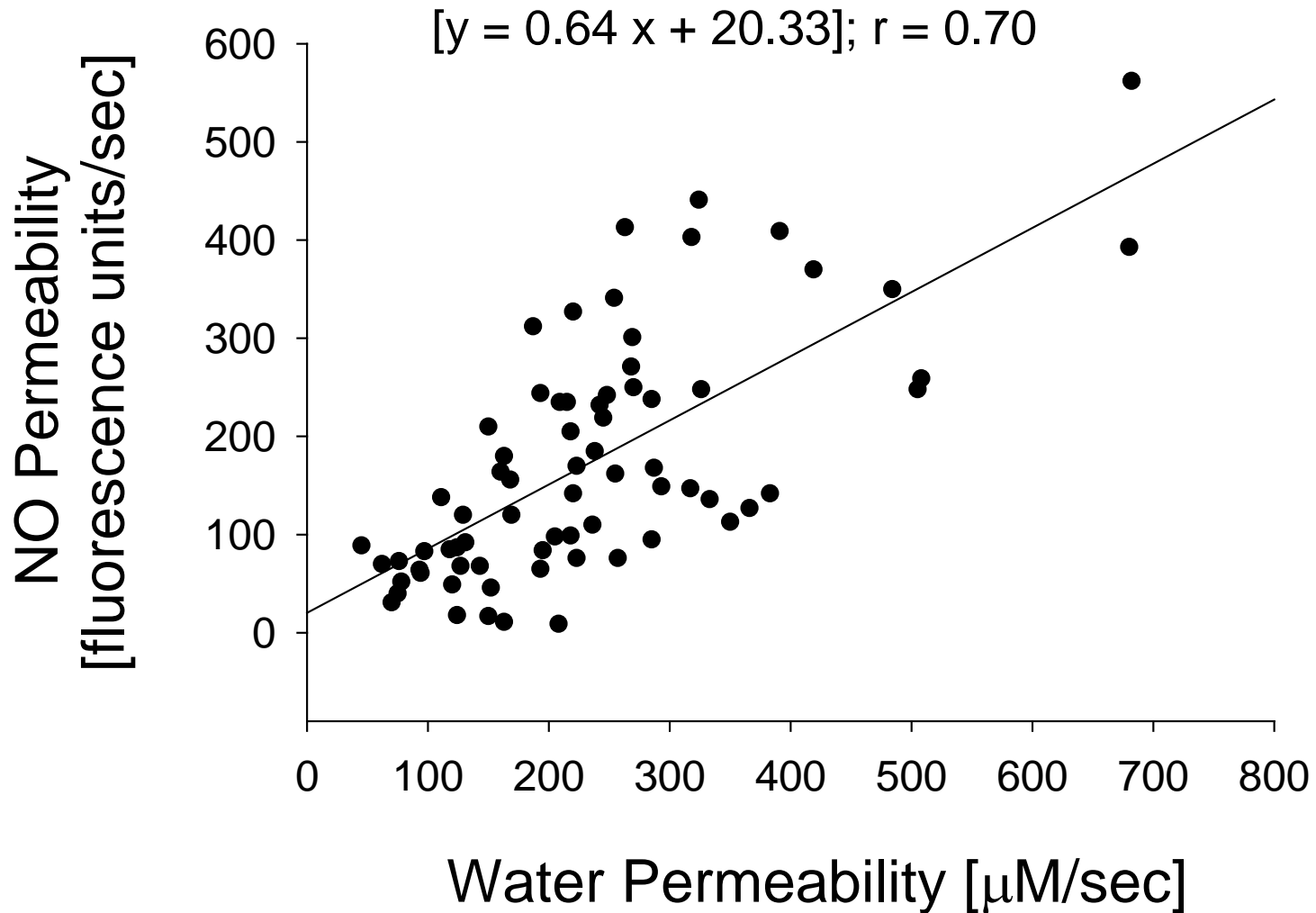
If our hypothesis is correct:

1. NO permeability (P_{NO}) should correlate with water permeability (P_f).
2. Increasing AQP-1 expression should increase NO flux.
3. Inhibitors of AQP-1 should reduce NO flux.
4. NO flux should be saturable.
5. Purified AQP-1 should transport NO.

Measuring NO with DAF in cultured cells

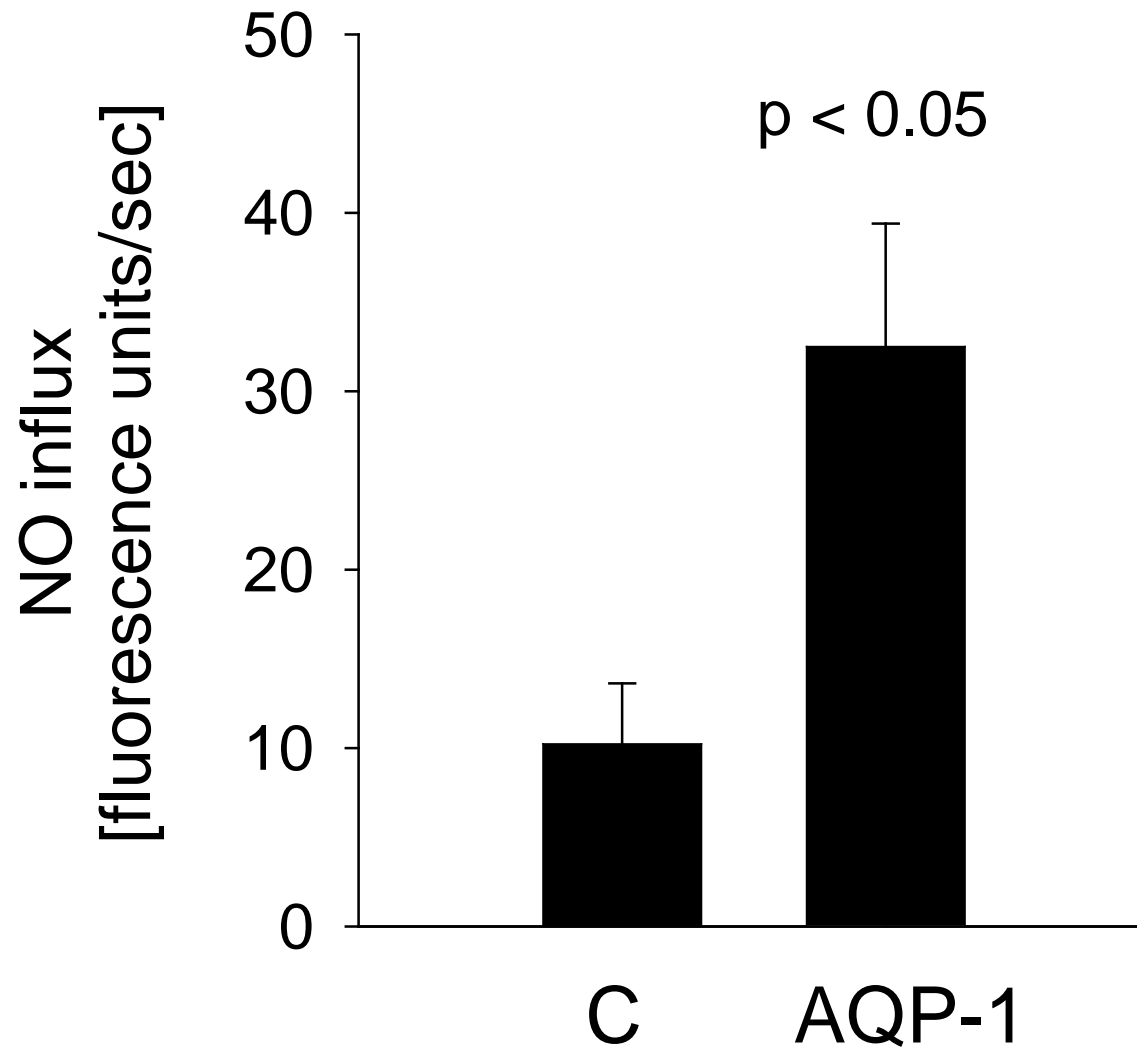


Correlation of P_{NO} and P_f in stably transfected CHO cells



1. NO permeability (P_{NO}) correlates with water permeability (P_f).
2. Increasing AQP-1 expression should increase NO flux.
3. Inhibitors of AQP-1 should reduce NO flux.
4. NO flux should be saturable.
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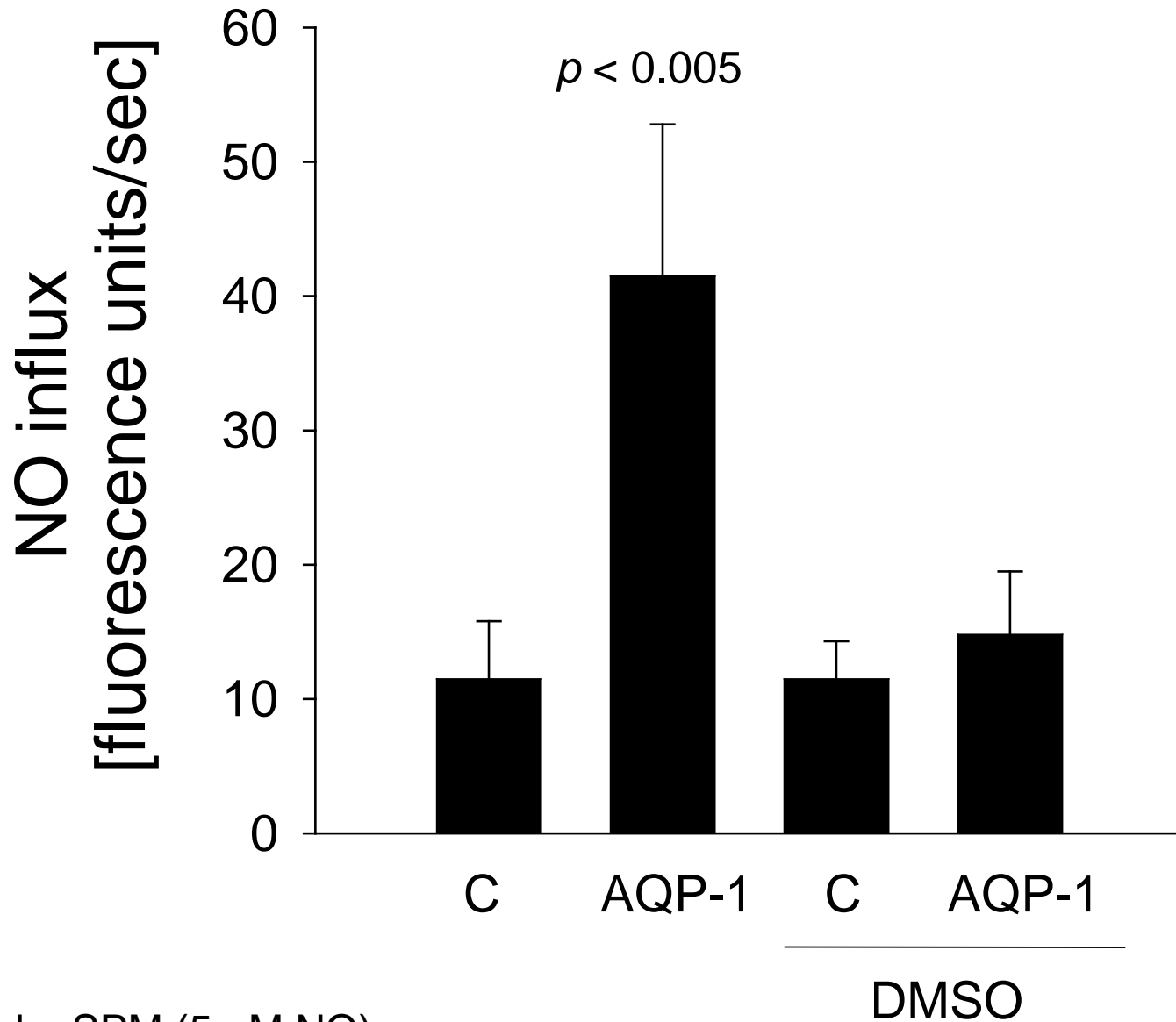
Effect of transiently transfecting CHO cells with aquaporin-1 (AQP-1) on NO influx



NO gradient by SPM (5 μ M NO)

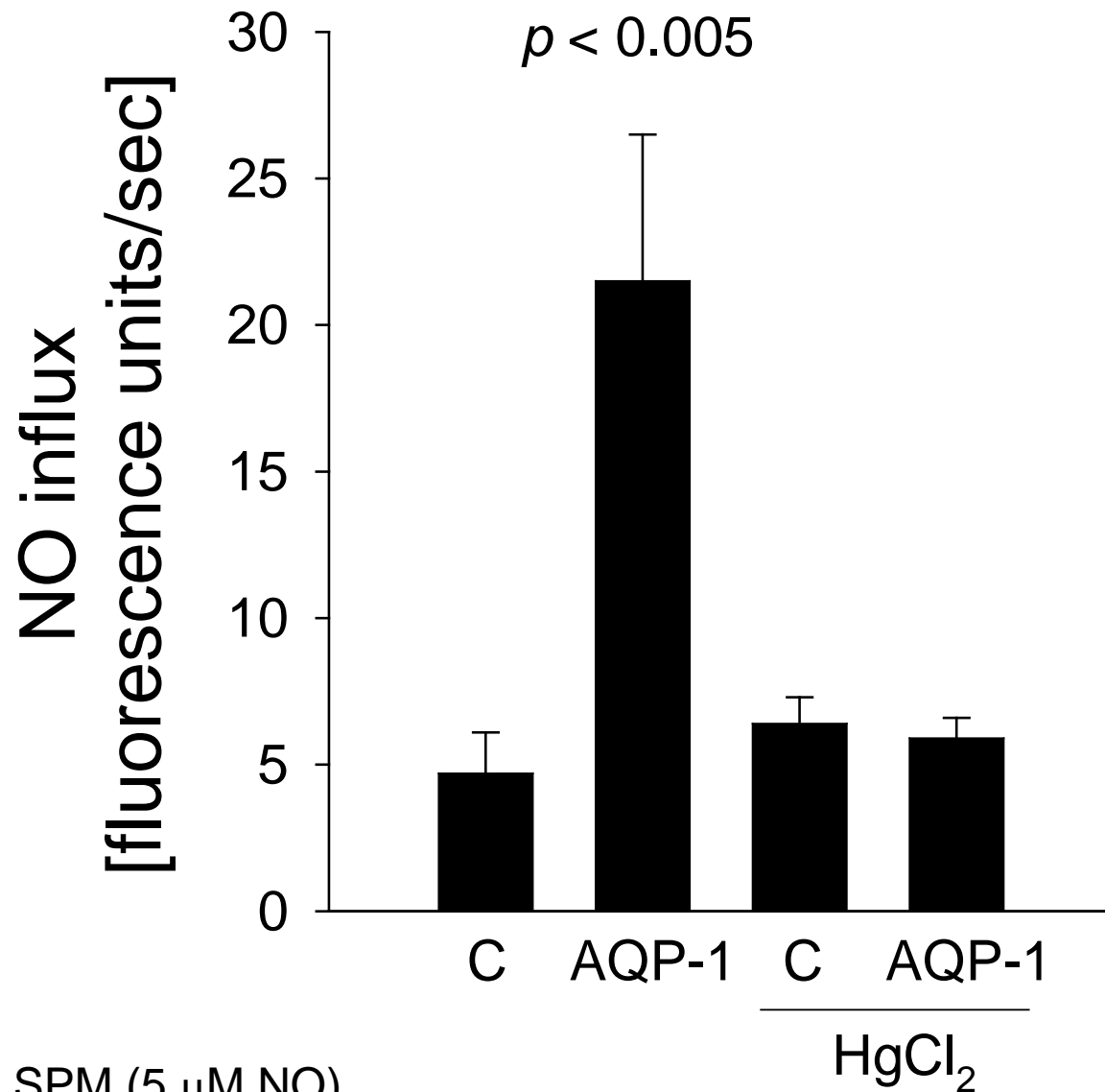
1. NO permeability (P_{NO}) correlates with water permeability (P_f).
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4. NO flux should be saturable.
5. Purified AQP-1 should transport NO.

Effect of DMSO, an AQP-1 inhibitor, on NO influx into transiently transfected CHO cells

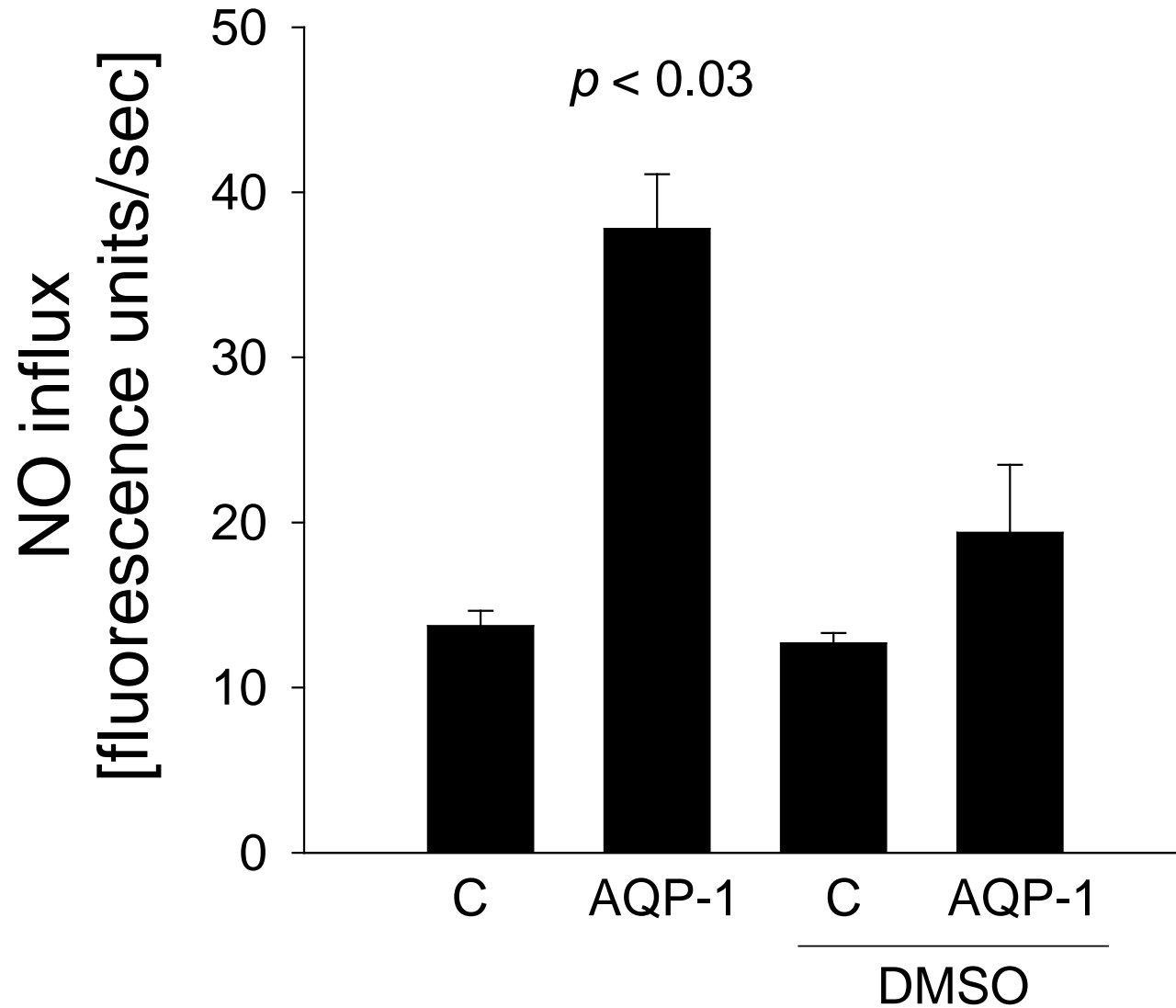


NO gradient by SPM (5 μ M NO)

Effect of 20 μM HgCl_2 , an AQP-1 inhibitor, on NO influx into transfected CHO cells



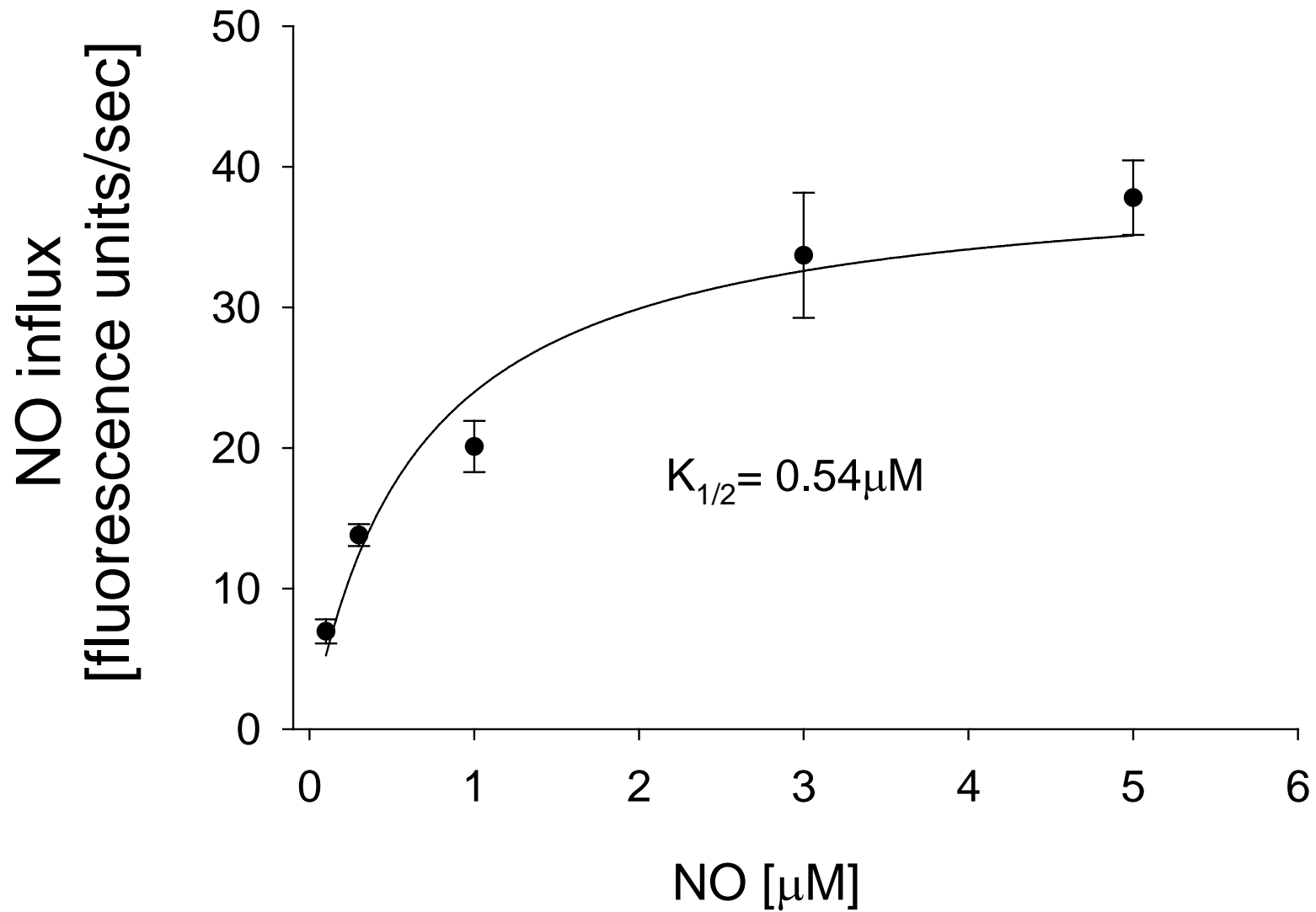
Effect of DMSO on NO influx into transiently transfected CHO cells



NO gradient by gas (5 μ M NO)

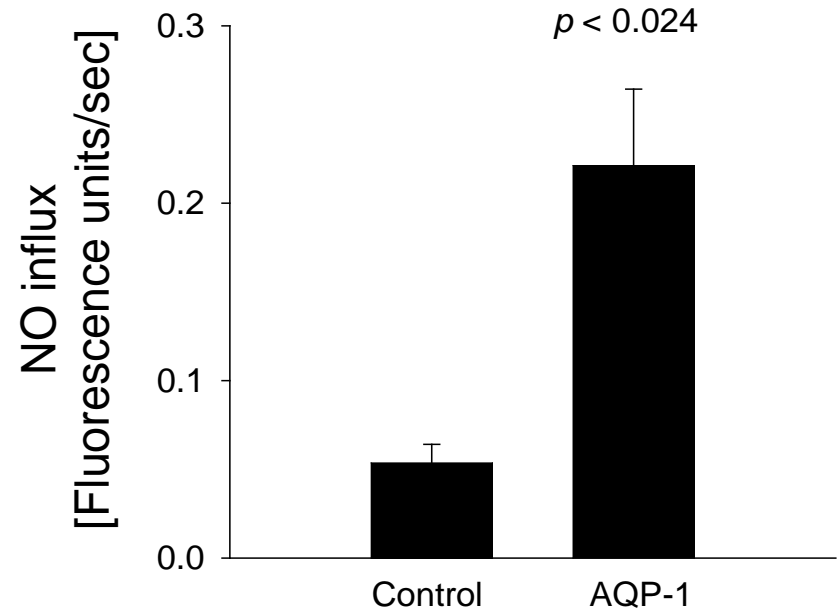
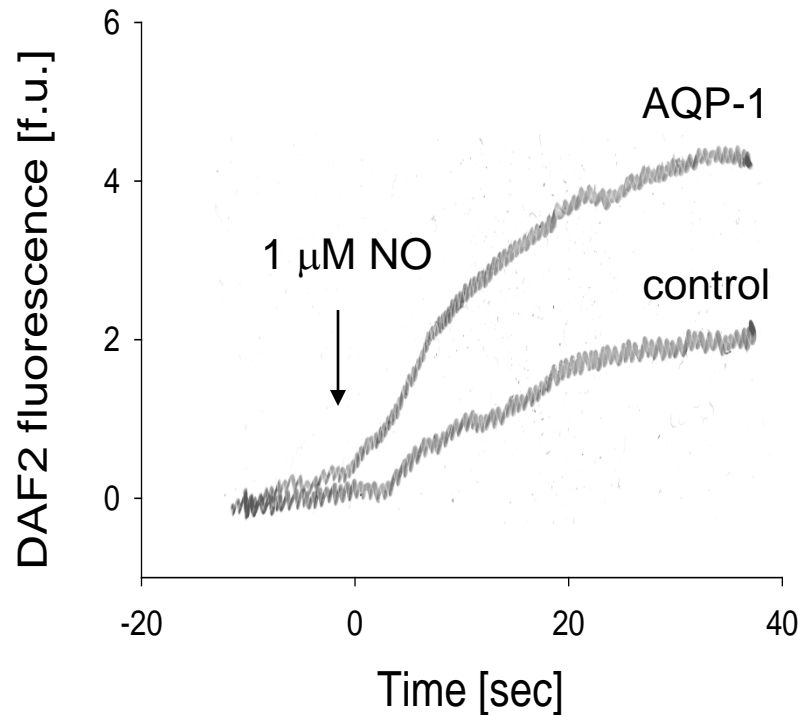
1. NO permeability (P_{NO}) correlates with water permeability (P_f).
2. Increasing AQP-1 expression increases NO flux.
3. Inhibitors of AQP-1 reduce NO flux.
4. NO flux should be saturable.
5. Purified AQP-1 should transport NO.

Concentration-dependent NO flux using NO gas



1. NO permeability (P_{NO}) correlates with water permeability (P_f).
2. Increasing AQP-1 expression increases NO flux.
3. Inhibitors of AQP-1 reduce NO flux.
4. NO flux is saturable.
5. Purified AQP-1 should transport NO.

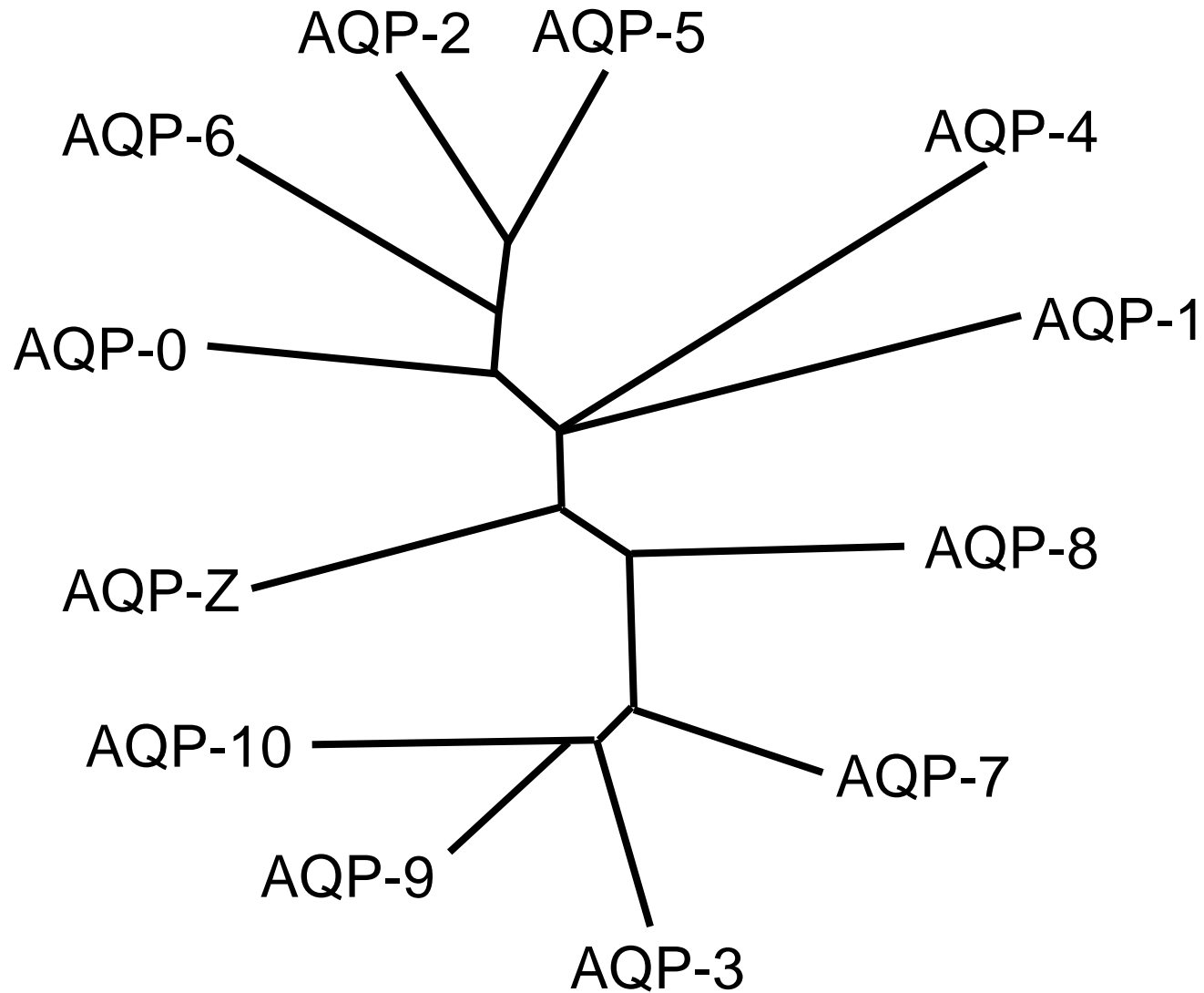
NO flux into proteoliposomes made with purified AQP-1



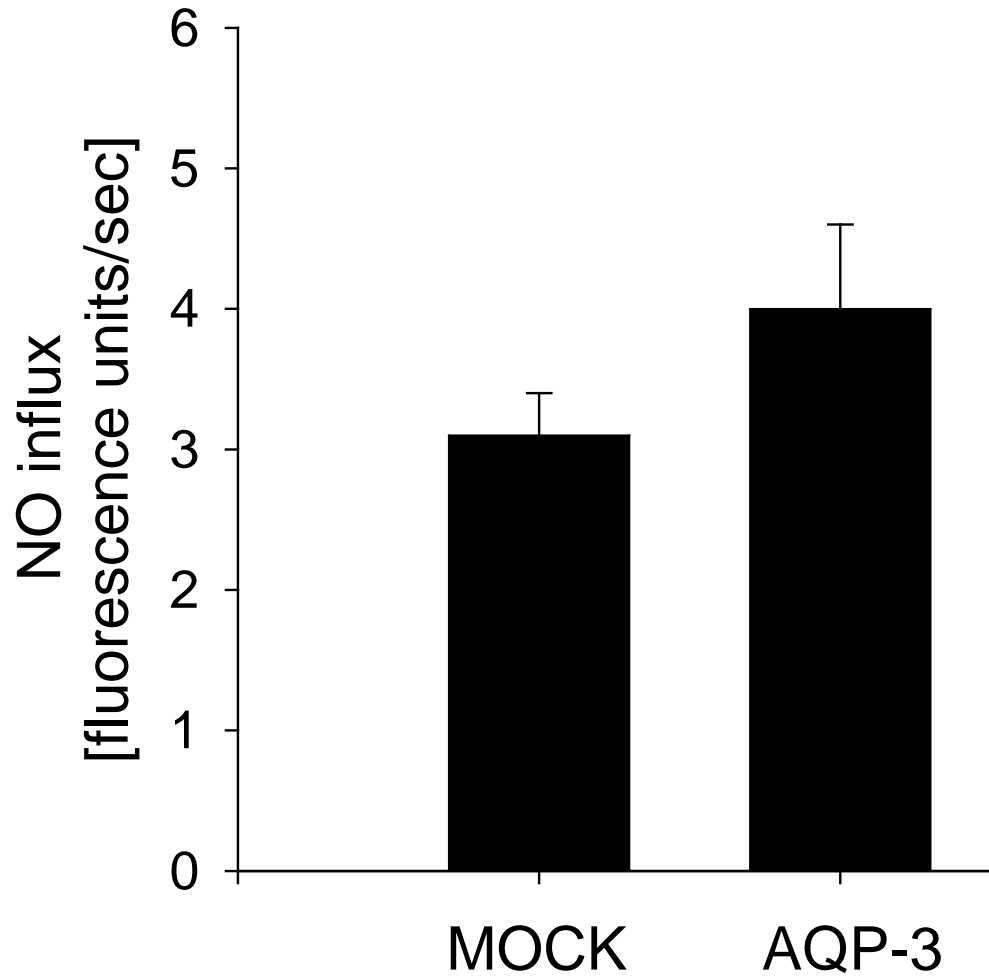
1. NO permeability (P_{NO}) correlates with water permeability (P_f).
2. Increasing AQP-1 expression increases NO flux.
3. Inhibitors of AQP-1 reduce NO flux.
4. NO flux is saturable.
5. Purified AQP-1 increases NO transport.

Do other aquaporins transport NO?

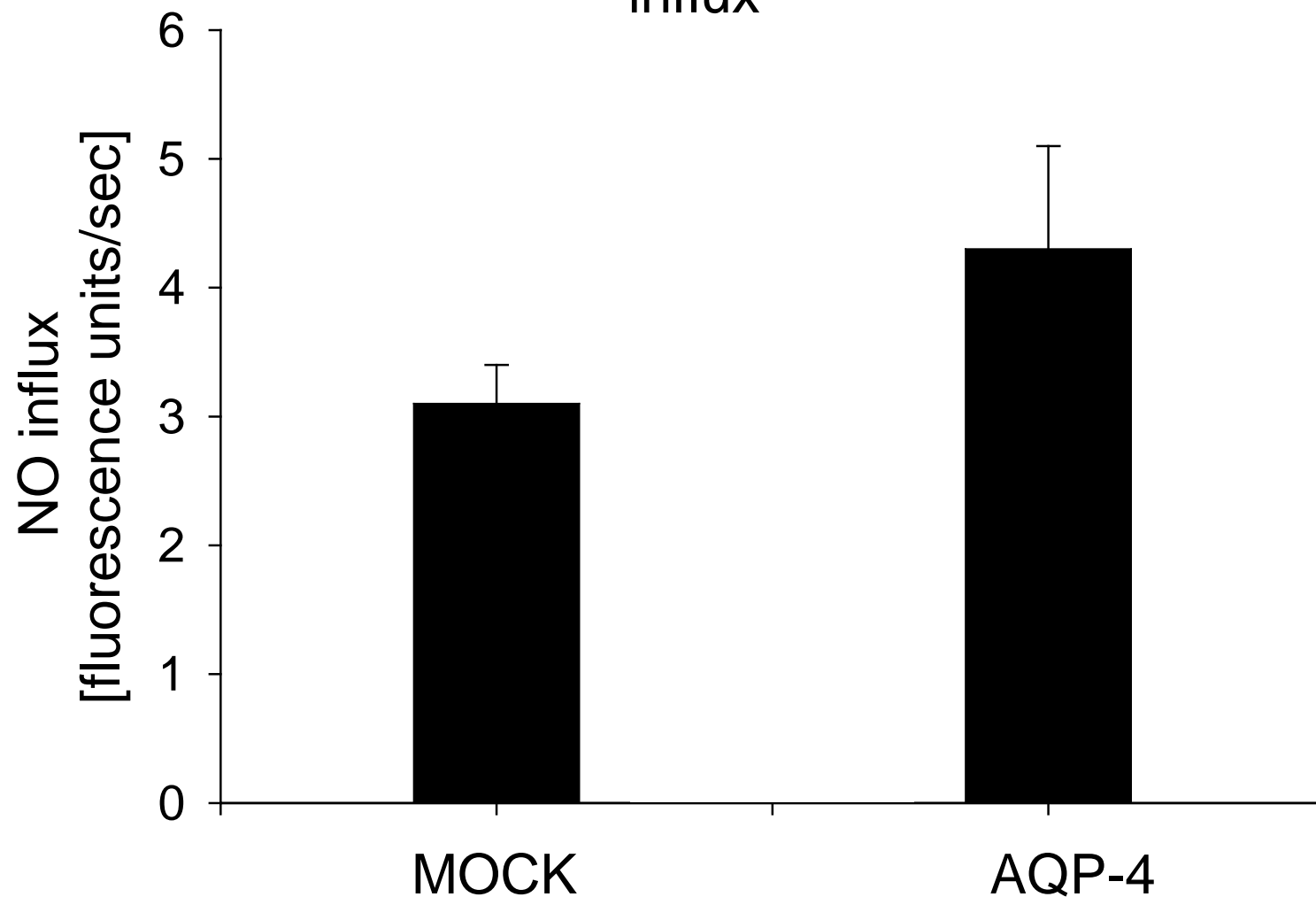
Partial aquaporin family tree



Effect of transiently transfecting CHO cells with AQP-3 on NO influx



Effect of transiently transfecting CHO cells with AQP-4 on NO influx

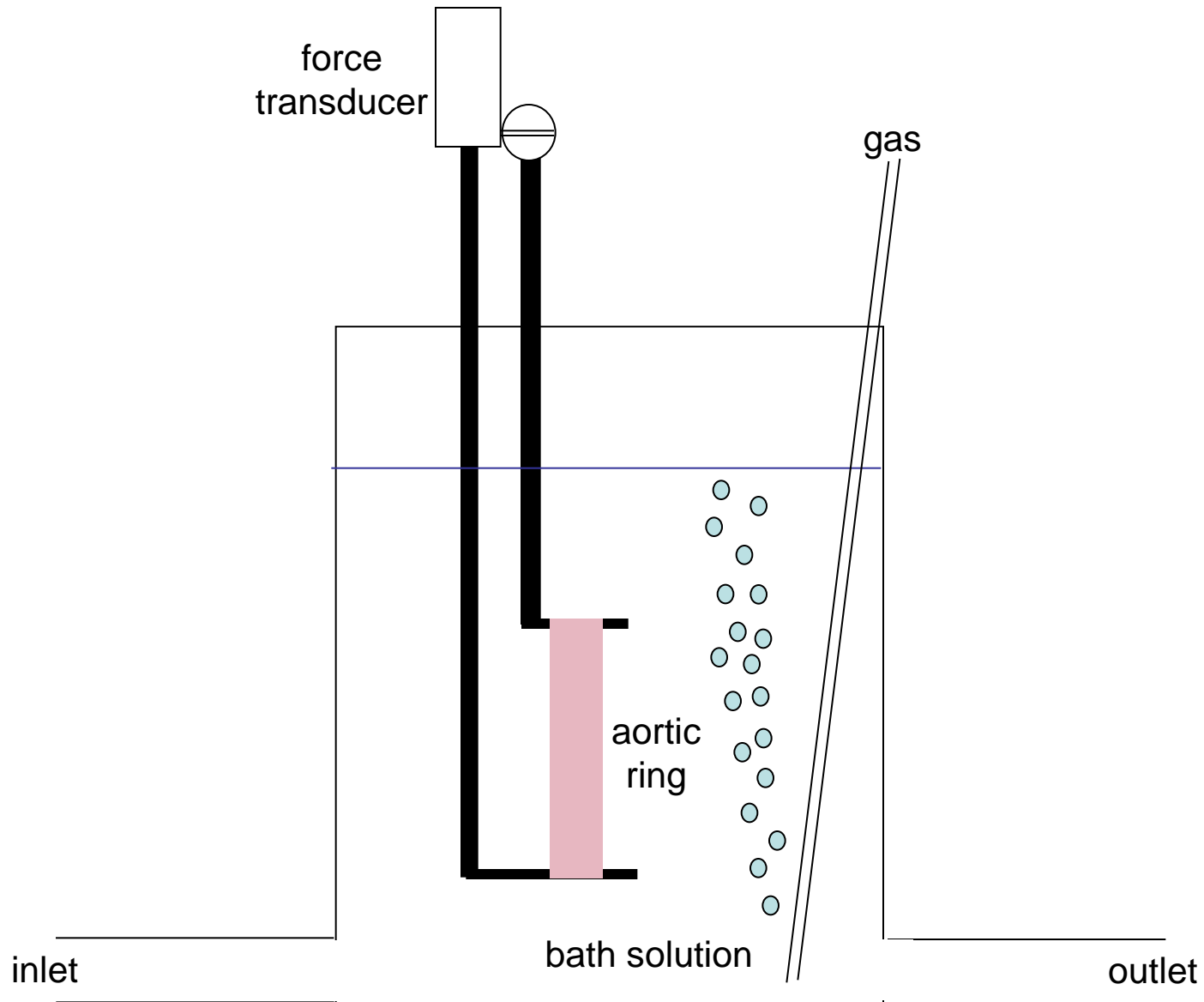


AQP-3 and AQP-4 may transport NO.
More data are required.

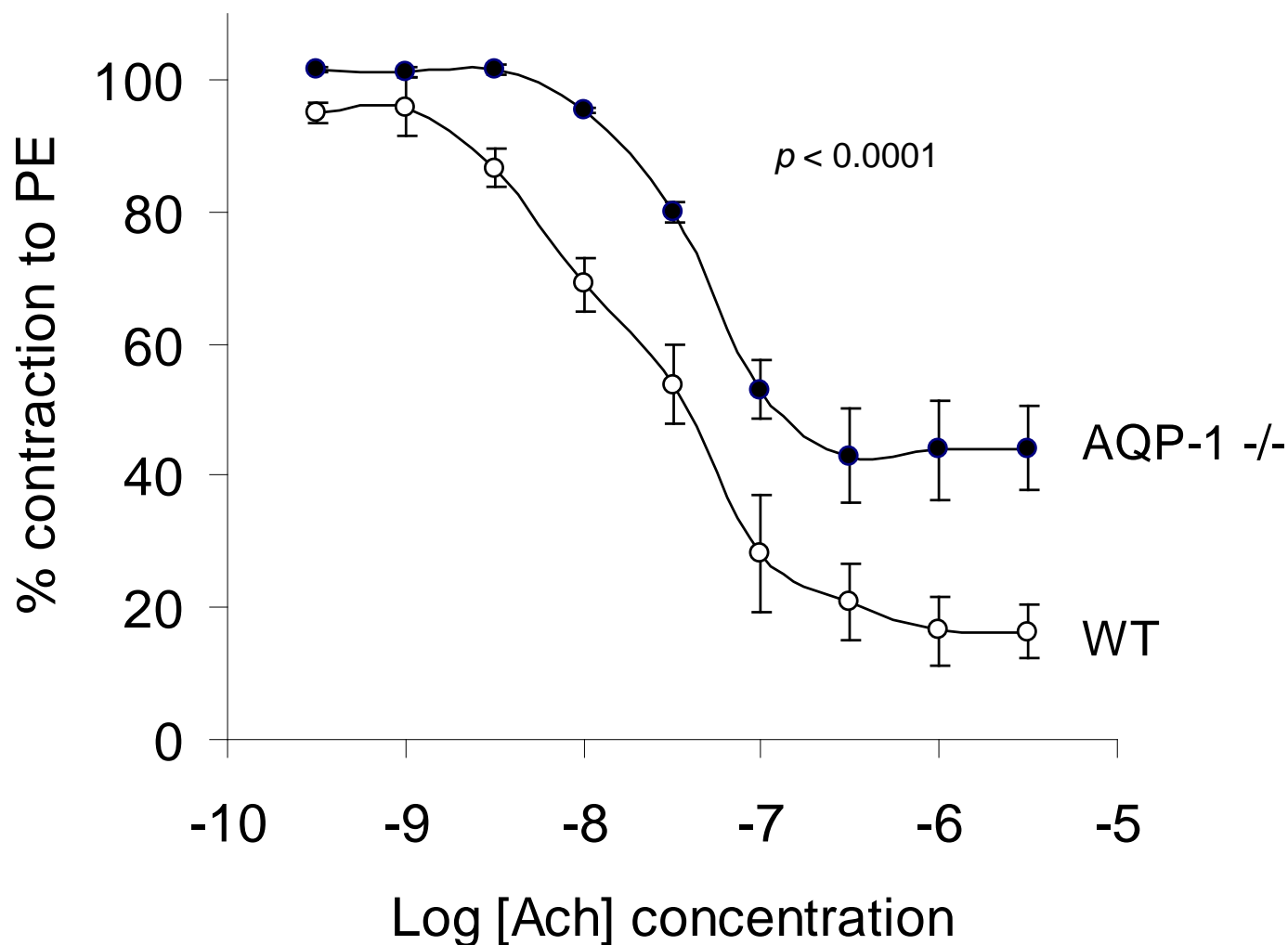
How does NO transport by AQP-1 compare to diffusion through the bilayer in “real” cells?

Is it physiologically significant?

Aortic ring preparation



Acetylcholine-dependent relaxation of aortic rings from wild type and AQP-1 $-/-$ mice



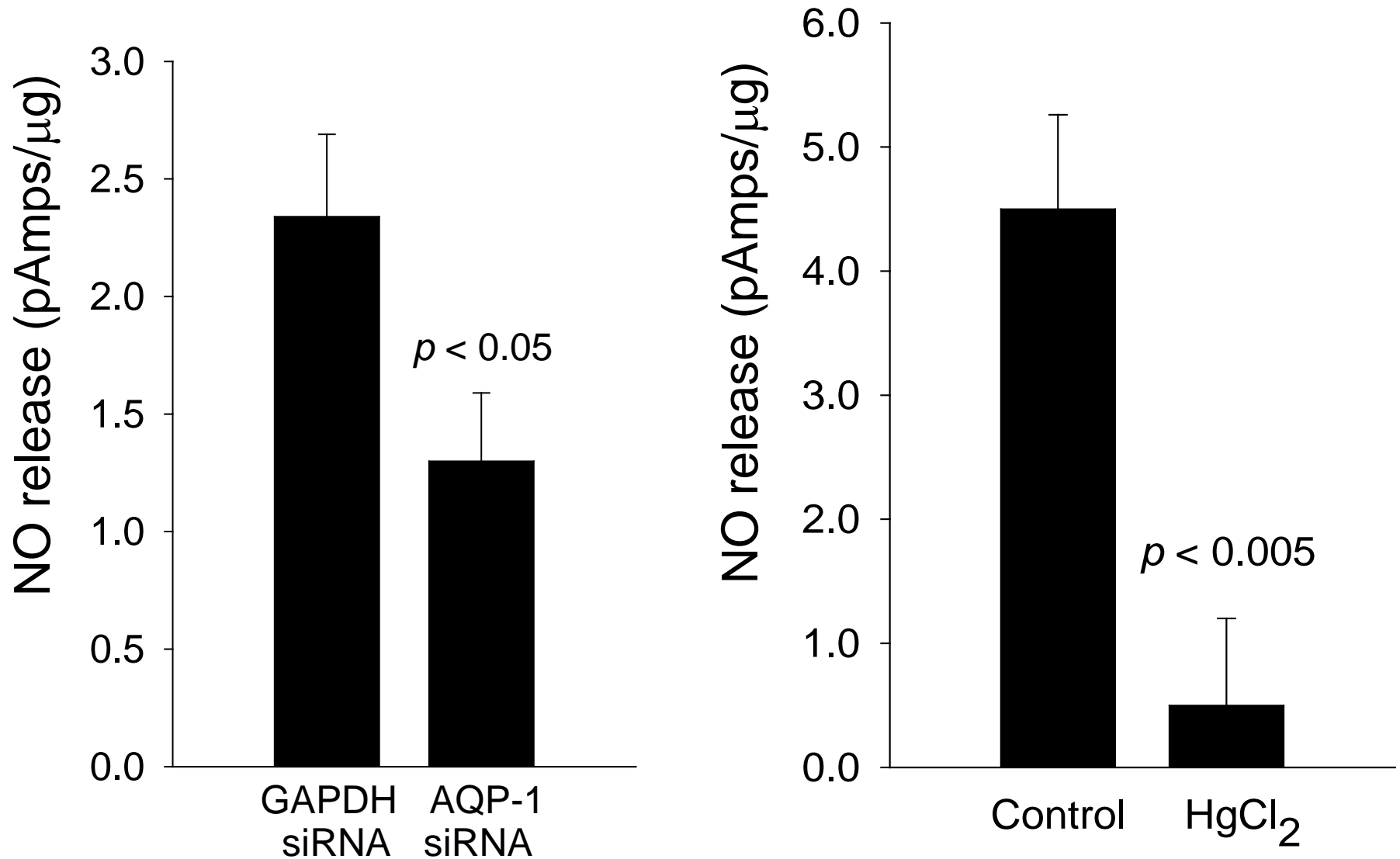
The reduction in Ach-induced relaxation in AQP-1 $-/-$ mice is NOT due to:

1. Less NOS 3. There is more in AQP-1 $-/-$ mice than WT.
2. Defective signaling down-stream of NO. Donors that release NO inside VSMCs and cGMP relax rings from AQP-1 $-/-$ mice the same as WT.

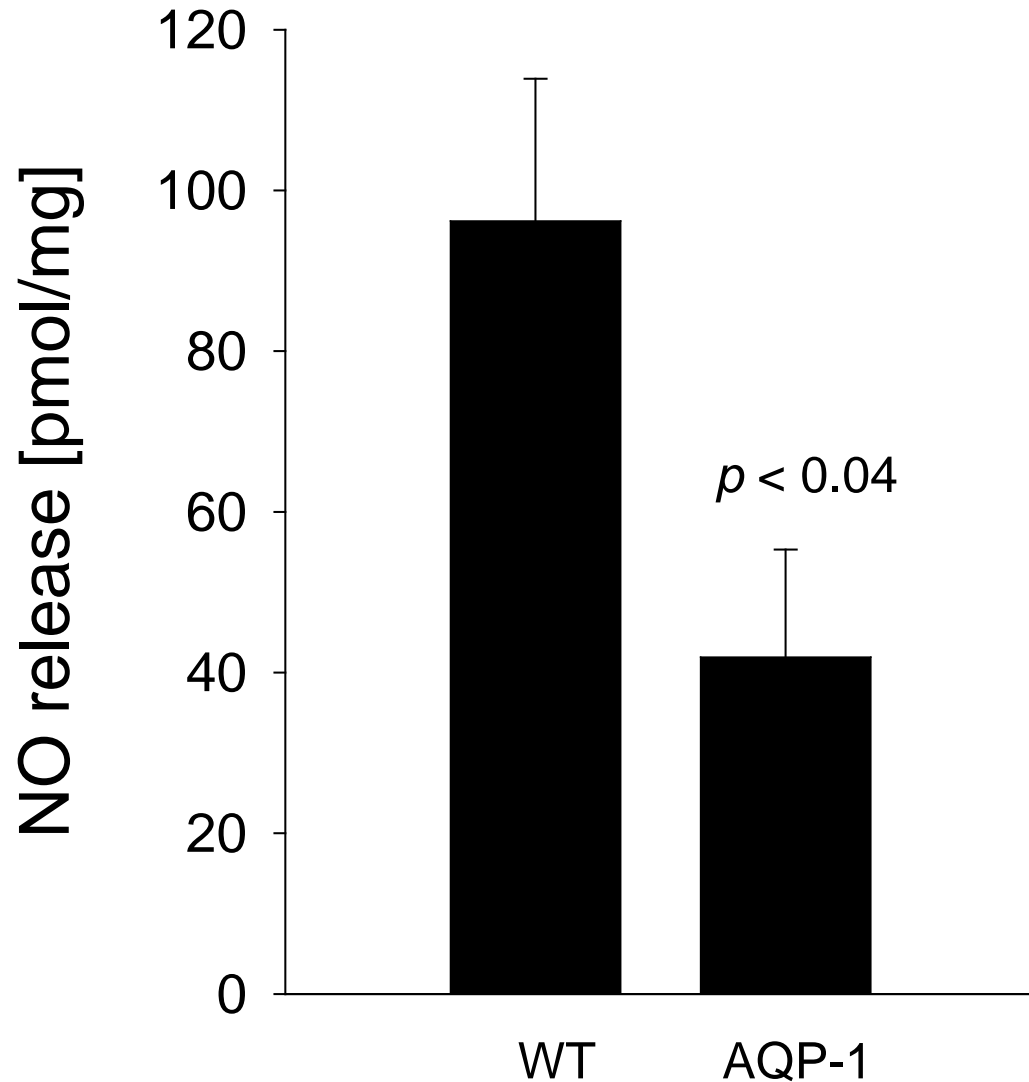
The reduction in Ach-induced relaxation in AQP-1 ^{-/-} mice could be due to:

1. Reduced NO efflux out of endothelial cells; and/or
2. Reduced NO influx into vascular smooth muscle cells.

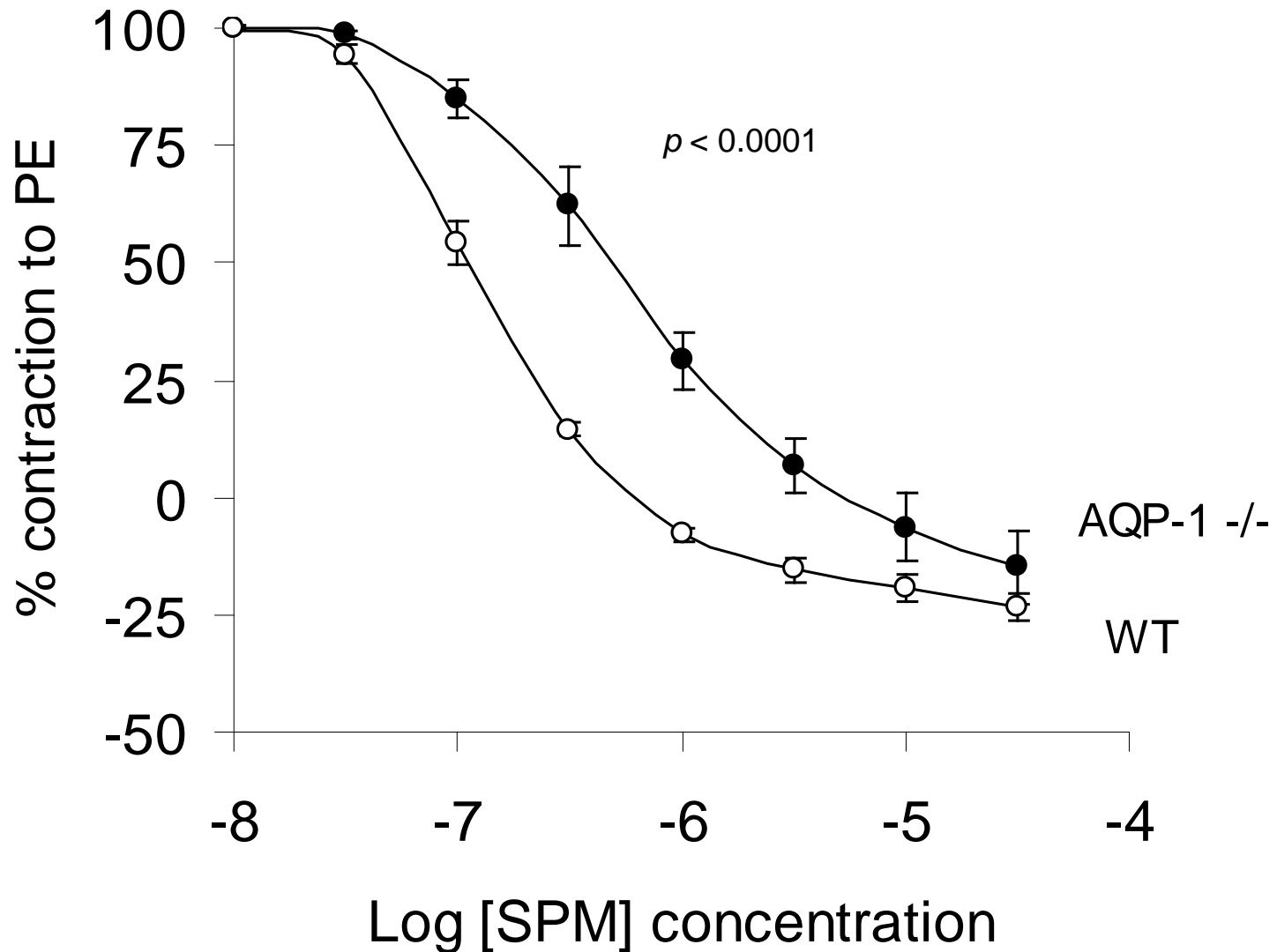
Effect of inhibiting AQP-1 on NO release by pancreatic endothelial cells



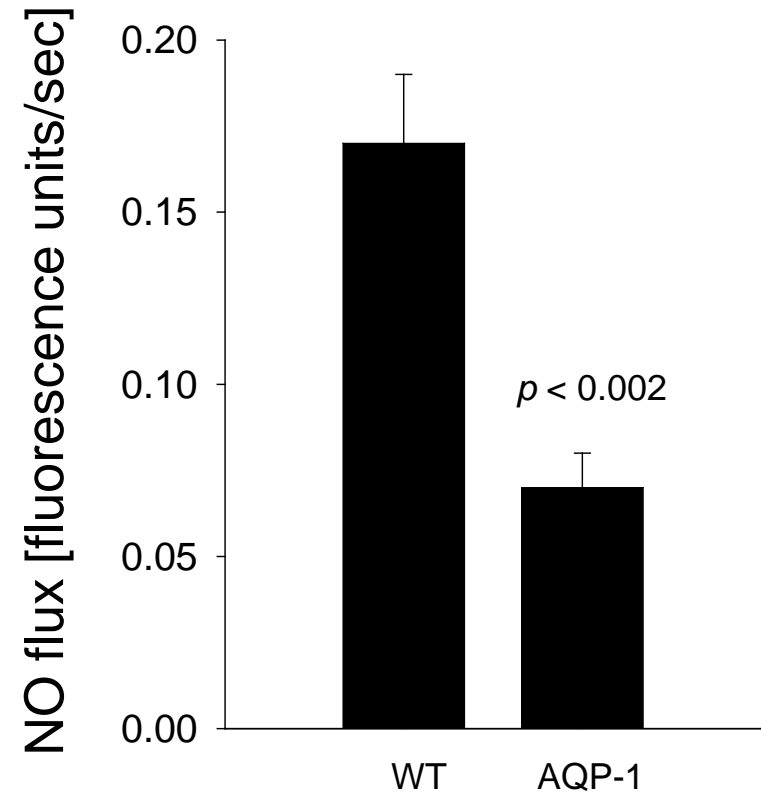
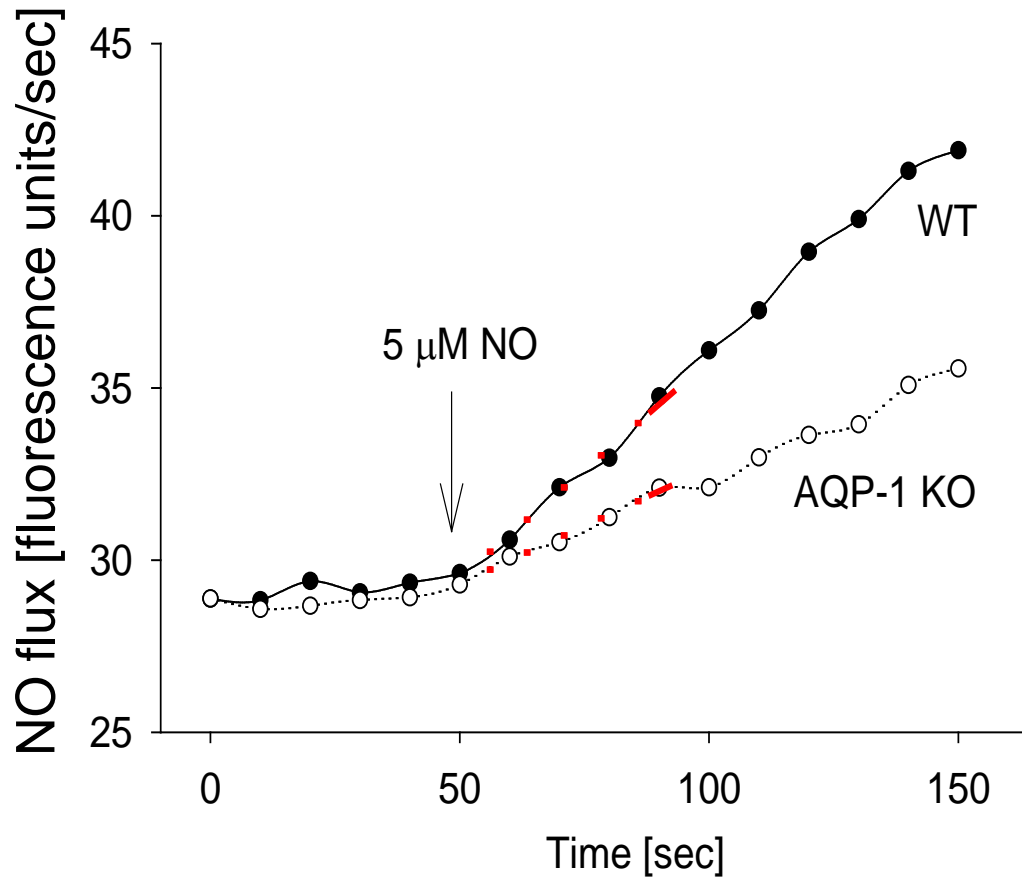
NO release by cultured aortic endothelial cells from wild type and AQP-1 -/- mice



Relaxation of denuded aortic rings to spermine NONOate, an NO donor that releases NO into the bathing media



NO influx into isolated aortic vascular smooth muscle cells from wild type and AQP-1 -/- mice



We are trying to show that the reduction in NO transport by AQP-1 is physiologically relevant in vivo by showing that total peripheral resistance does not decrease in response to acetylcholine in these mice as much as wild type mice

BUT

it seems that these mice have compensation mechanism including increased prostaglandin production and NOS expression that has frustrated our attempts thus far.

Conclusion

1. AQP-1 transports NO.
2. Transport of NO by AQP-1 occurs faster than by diffusion through the bilayer by about a factor of 2.
3. Transport of NO by AQP-1 appears to be physiologically significant.
4. Reduced Ach-dependent relaxation of aortic rings from AQP-1 $-/-$ mice is due to both reduced efflux out of endothelial cells and reduced influx into vascular smooth muscle cells.

***Role of Rh glycoproteins in NH₃ gas transport –
lessons from in vivo model
systems***

David Weiner, M.D.

University of Florida College of Medicine
and NF/SGVHS

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University of Florida Collaborators

Bill Clapp, M.D.

Byron Croker, M.D., Ph.D.

Kirsten M. Madsen, M.D.

R. Tyler Miller, M.D.

Clinical fellows:

Allen Vander, M.D.

Tom DelGiorno, M.D.

Kevin Schroeder, M.D.

Ian Steele, M.D.

Dietrich Werner, M.D.

New Zealand

Rob Walker, M.D.

Jennifer Bedford, Ph.D.

John Leader, Ph.D.

Ewha Womans University, Seoul, Korea

Ki-Hwan Han, M.D., Ph.D.

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Connie M. Westhoff, Ph.D.

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Janet Klein, Ph.D.

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Tekla Smith



Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Is renal collecting duct NH_3 transport diffusive or transporter-mediated?



Are Rh glycoproteins present in cells that transport NH_3 gas?



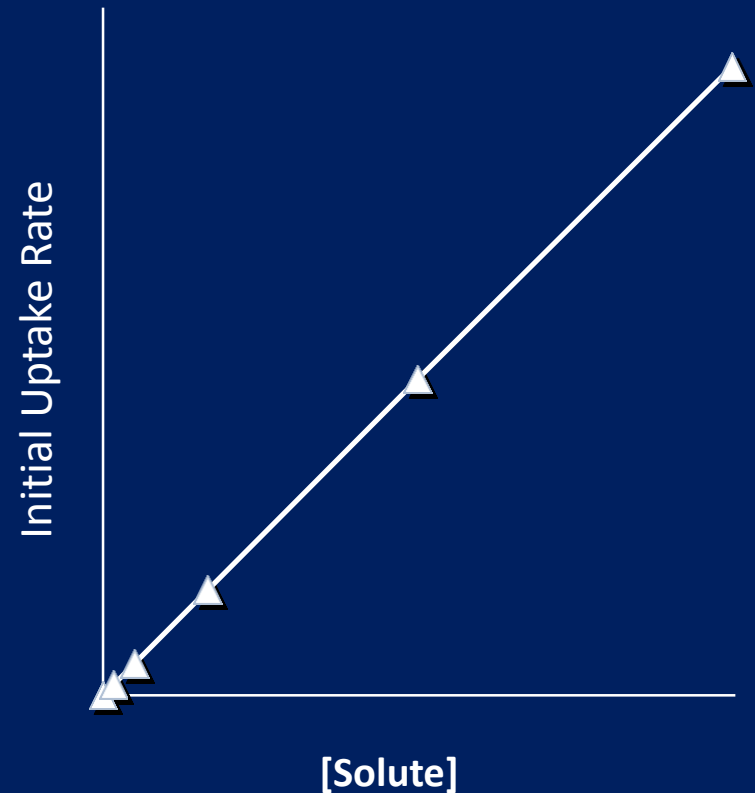
Does expression parallel changes in NH_3 gas transport?



Does Rh glycoprotein inhibition alter NH_3 gas transport?

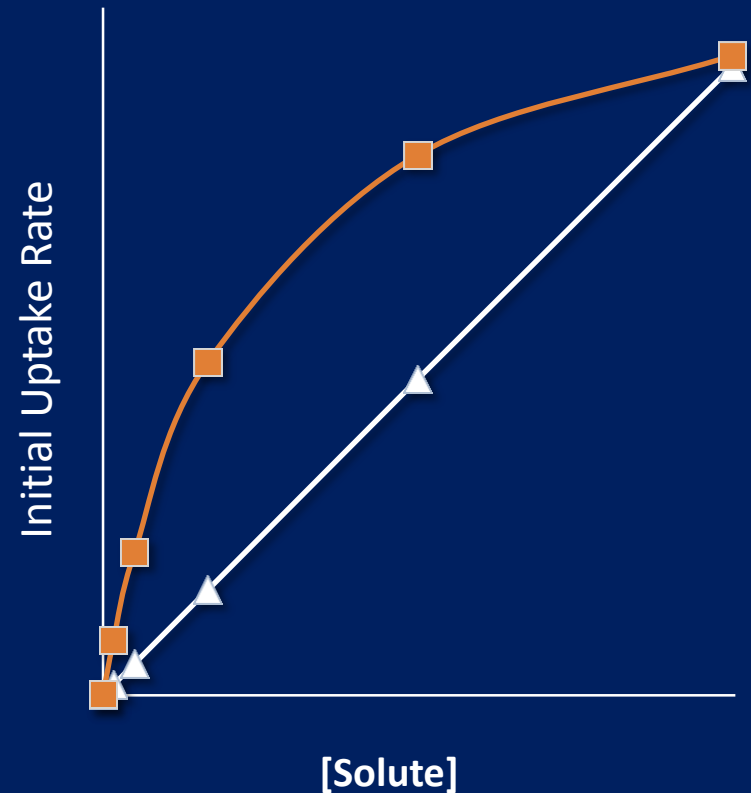
How can we determine whether collecting duct NH_3 transport is diffusive or transporter-mediated?

- Inhibitors
- Gene knock-down
- Functional tests
 - Diffusive
 - ◆ Transport proportional to concentration gradient

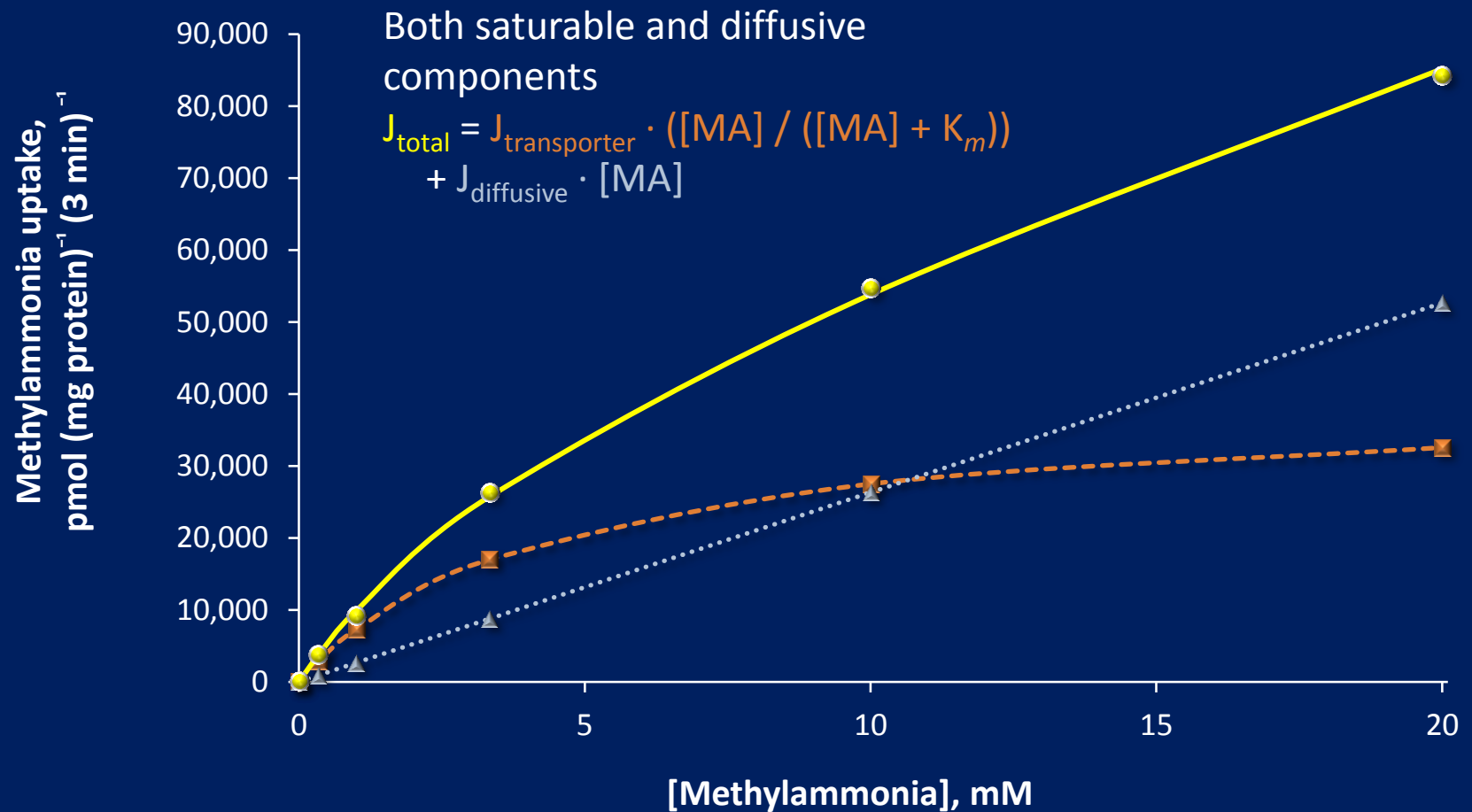


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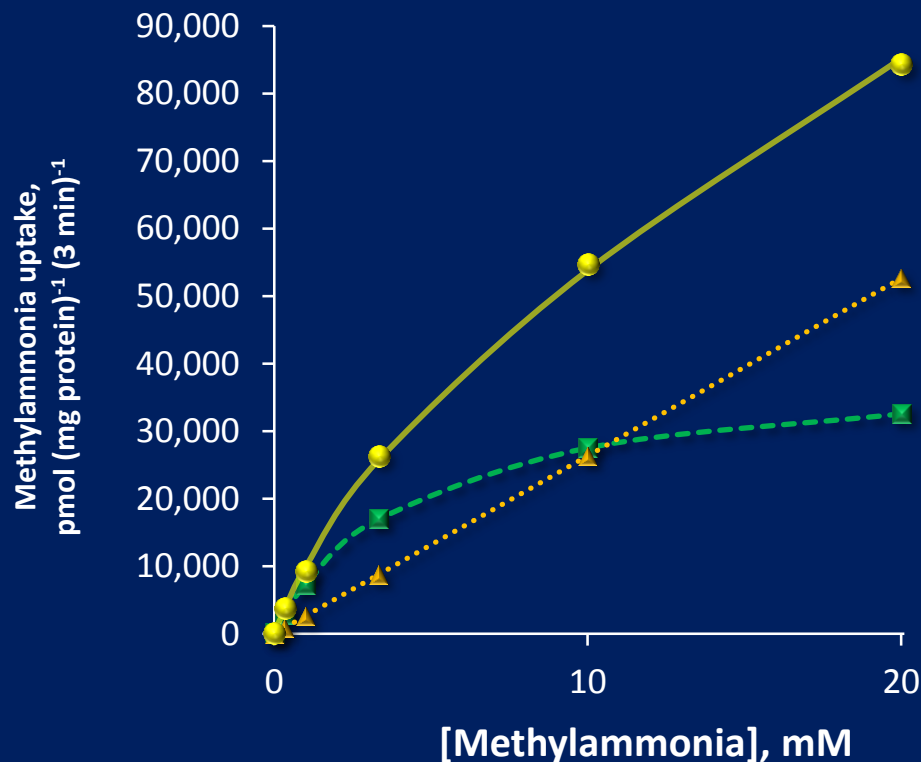
- Inhibitors
- Gene knock-down
- Functional tests
 - Diffusive
 - ◆ Transport proportional to concentration gradient
 - Transporter-mediated
 - ◆ **Saturable**



Measurement of collecting duct cell (mIMCD-3) basolateral total ammonia transport



Characteristics of collecting duct cell (mIMCD-3) basolateral membrane total ammonia transport



- Functional characteristics
 - Electroneutral
 - Na⁺ and K⁺-independent
 - Not inhibited by K⁺ transporter or NHE inhibitors
 - Extracellular and intracellular pH dependent
 - NH₃ transport
- Similar findings when studying apical transport
- Similar findings in gastric, hepatic, small intestinal and colonic epithelial cells

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Renal collecting duct NH_3 transport is both diffusive and saturable



Are Rh glycoproteins present in cells that transport NH_3 gas?

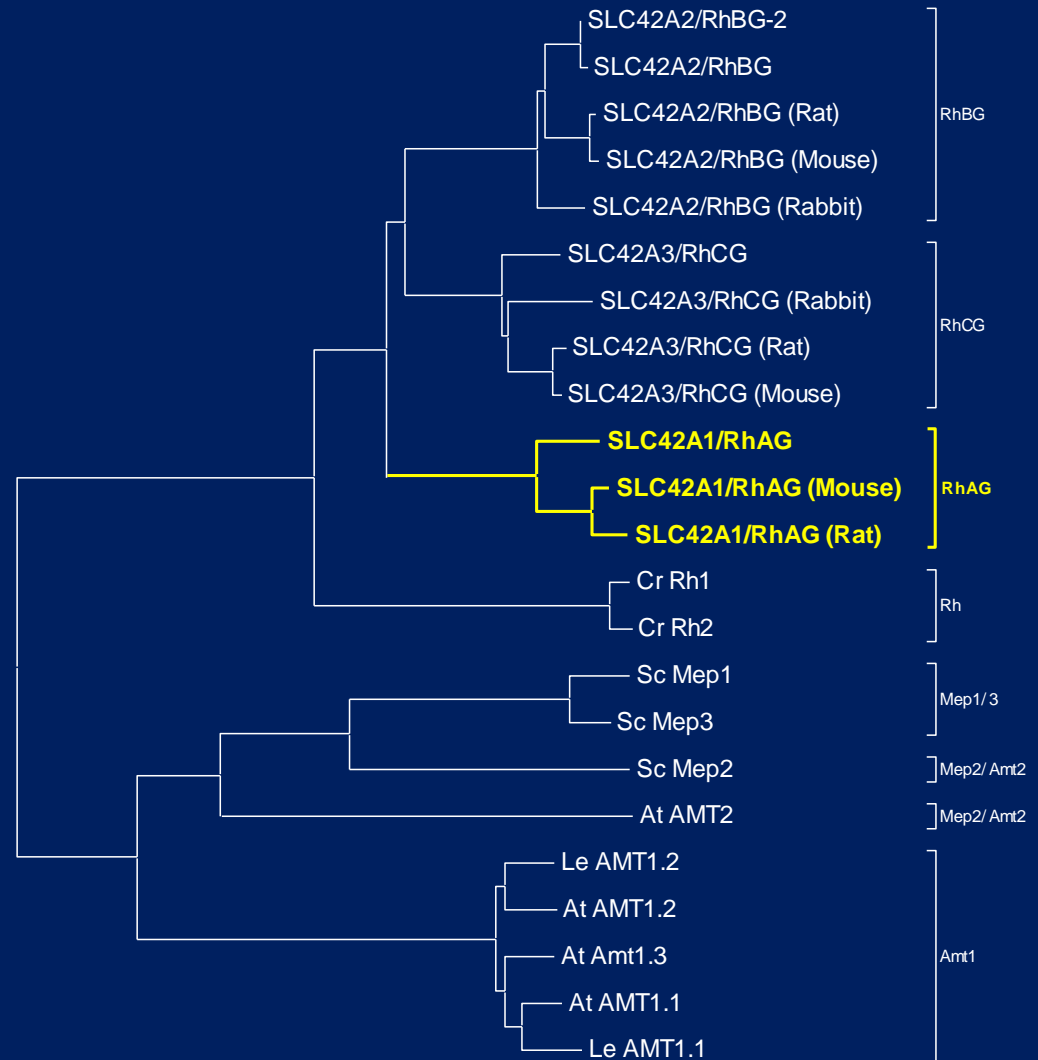
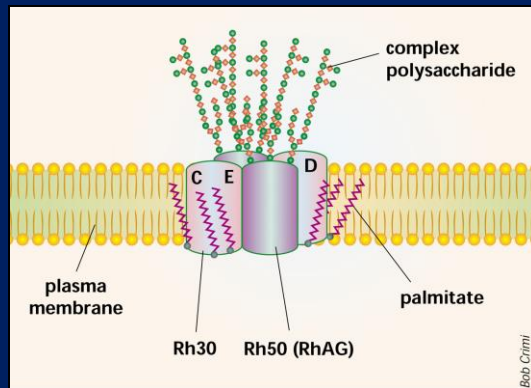


Does expression parallel changes in NH_3 gas transport?



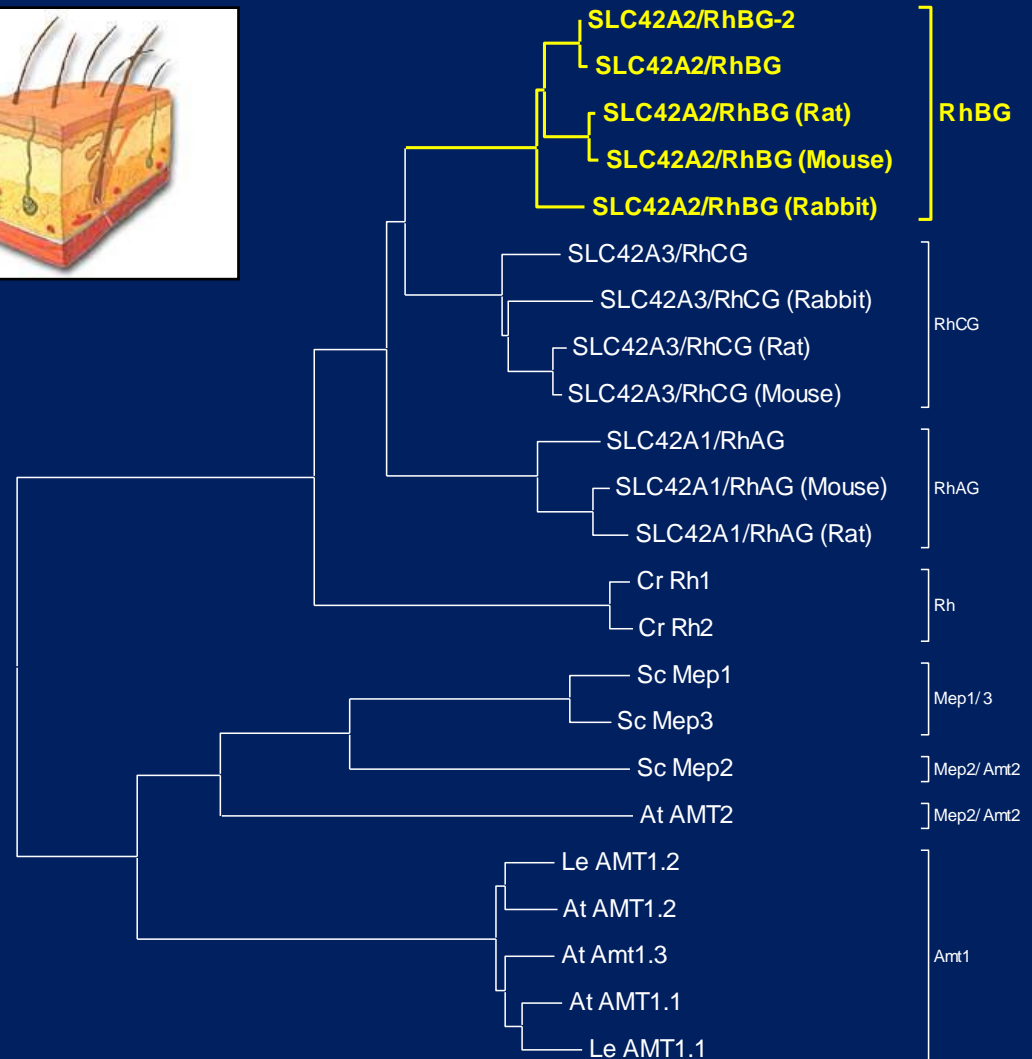
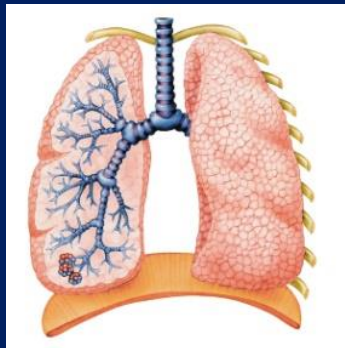
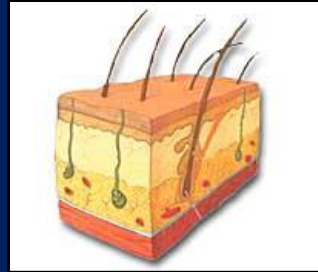
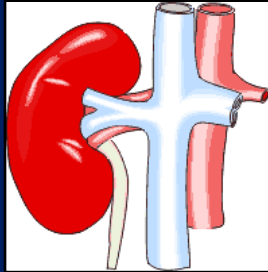
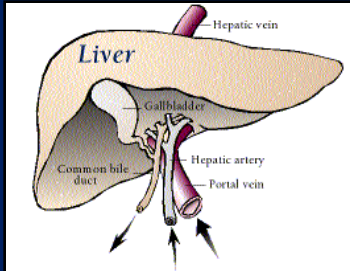
Does Rh glycoprotein inhibition alter NH_3 gas transport?

Where is RhAG/Rhag expressed?



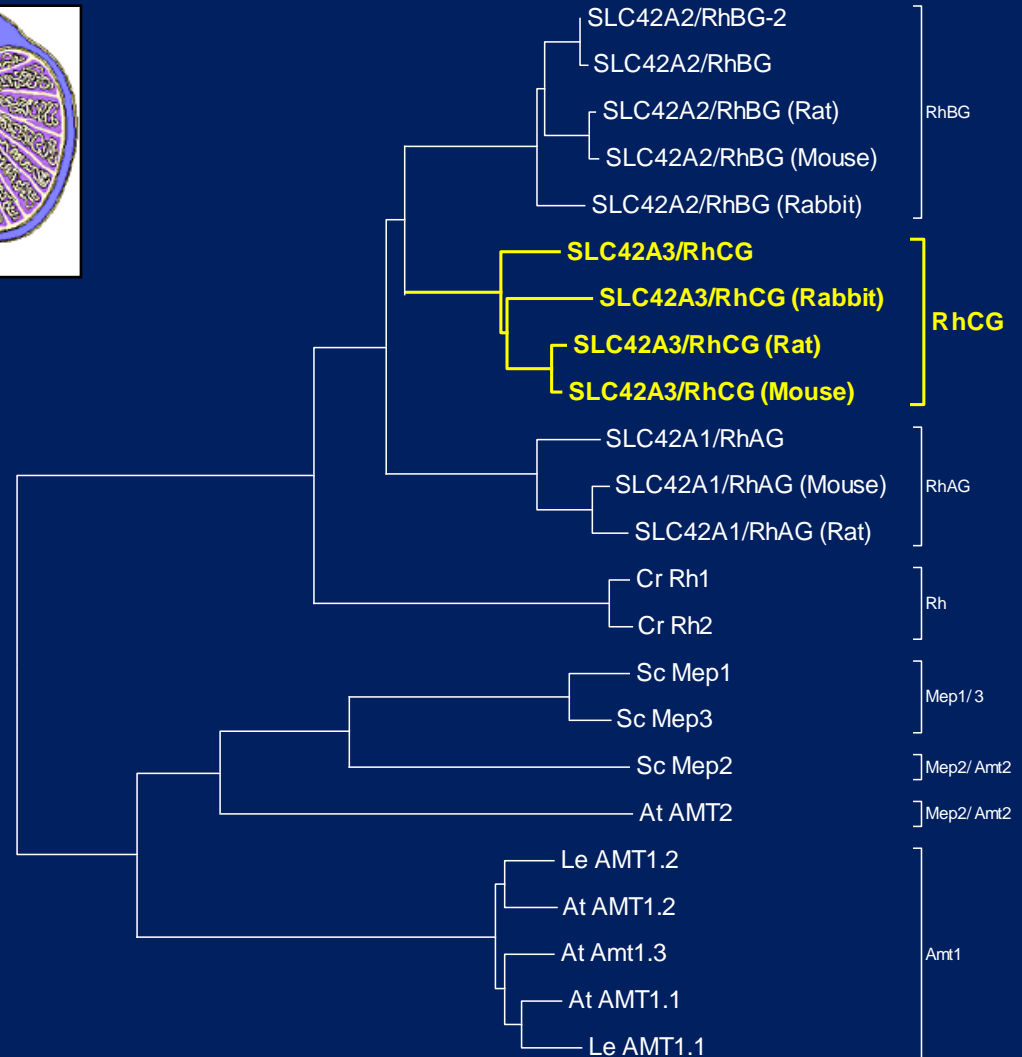
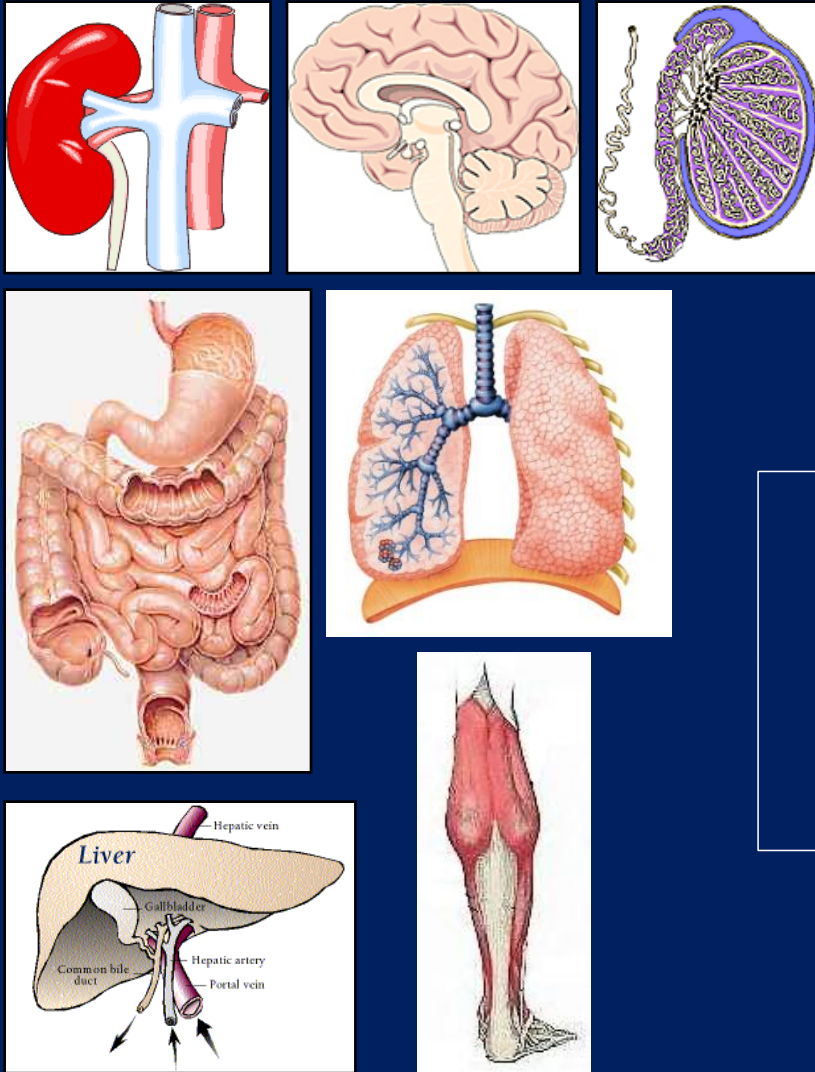
0.2

Where is Rhbg expressed?

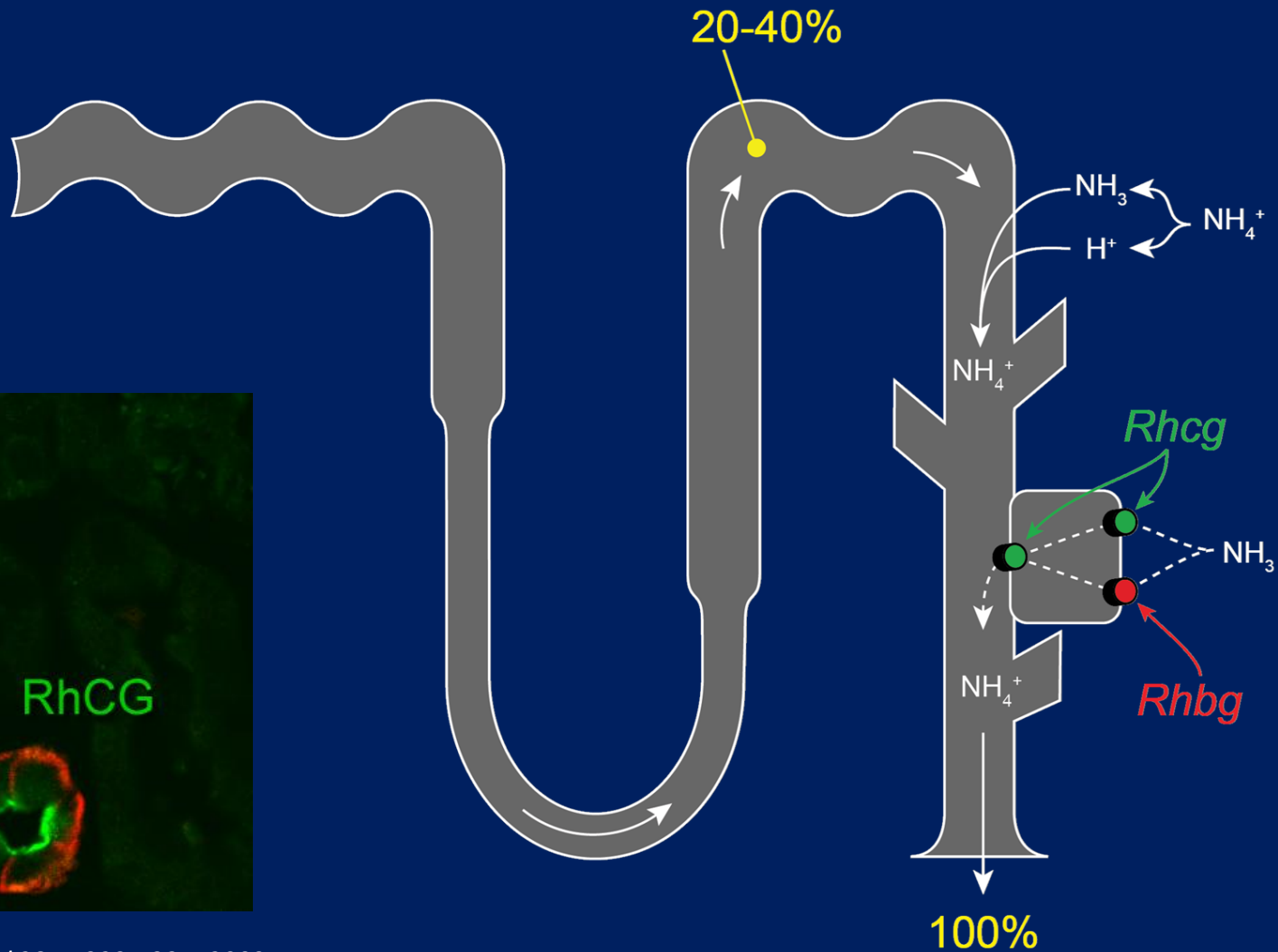
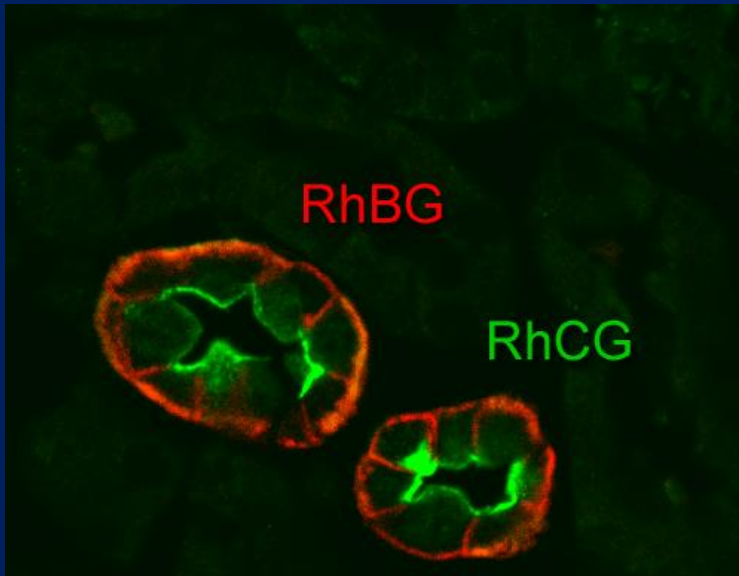


0.2

Where is Rhcg expressed?

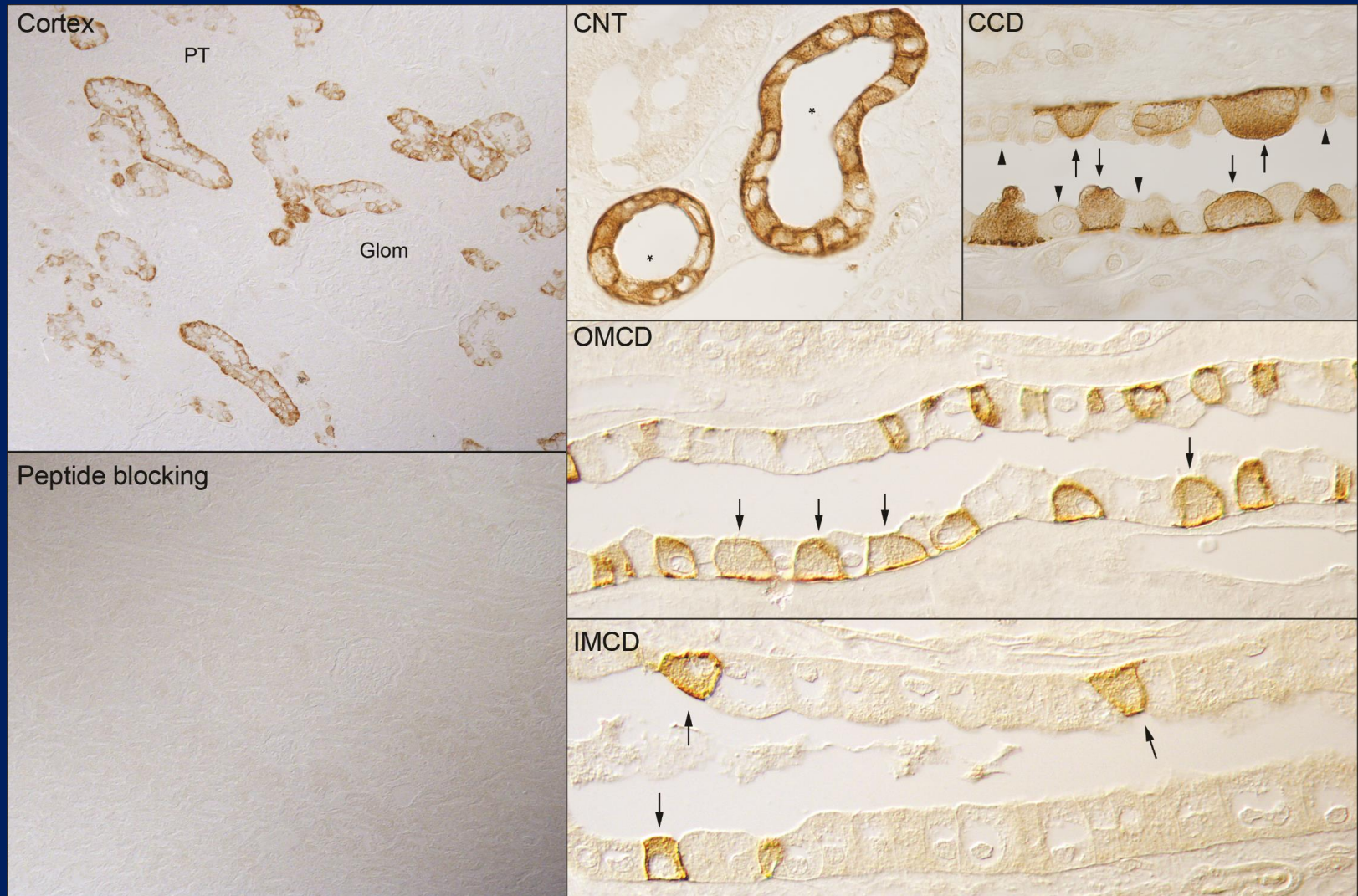


Renal Rh glycoprotein expression



JW Verlander, et al, *AJP Renal* 284:F323-F337, 2003.

RhCG expression in human kidney



Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Renal collecting duct NH_3 transport is both diffusive and saturable



Rh glycoproteins are present specifically in cells that transport NH_3

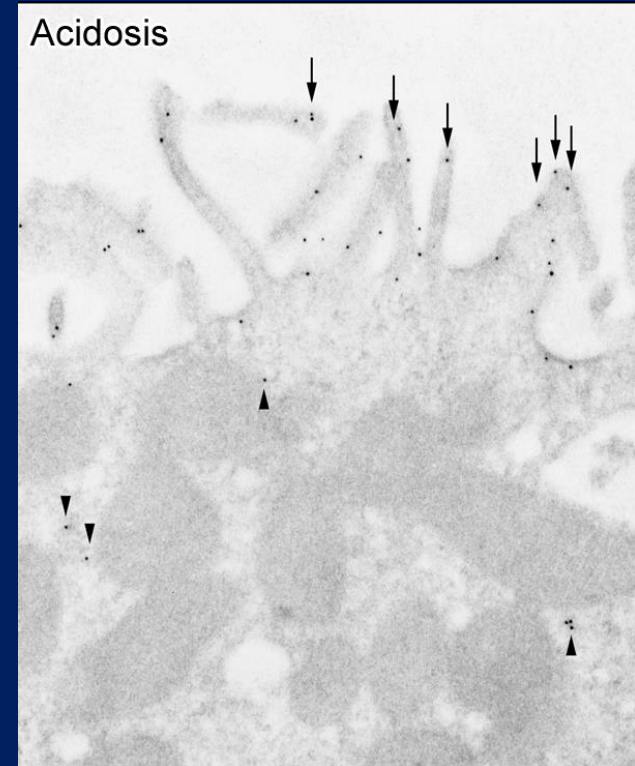
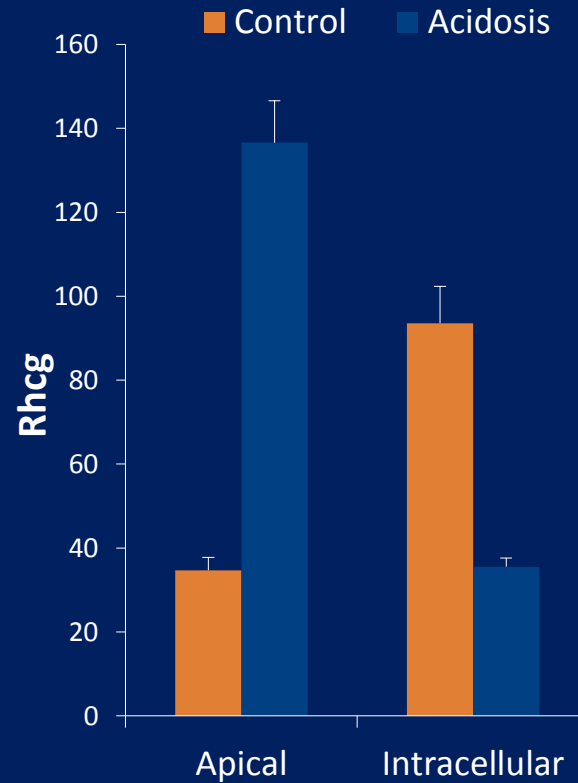
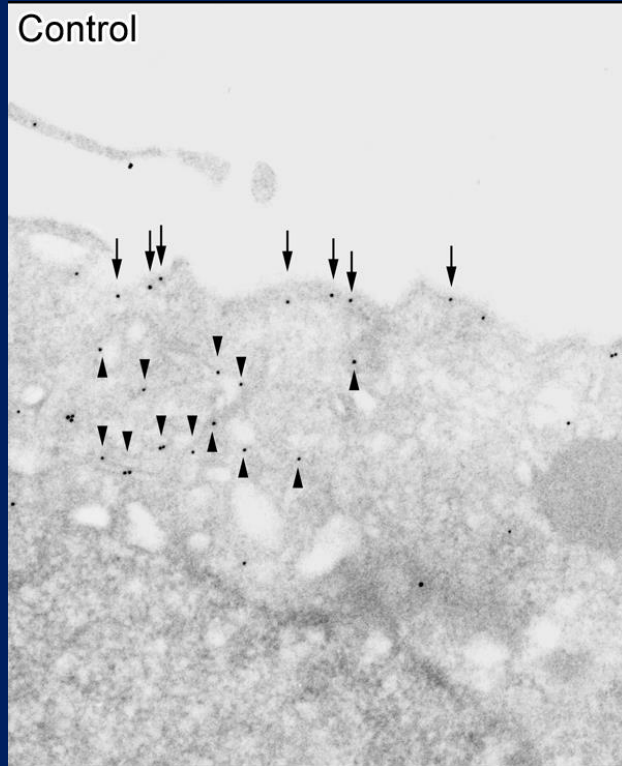


Does expression parallel changes in NH_3 gas transport?



Does Rh glycoprotein inhibition alter NH_3 gas transport?

Metabolic acidosis increases apical plasma membrane Rhcg expression

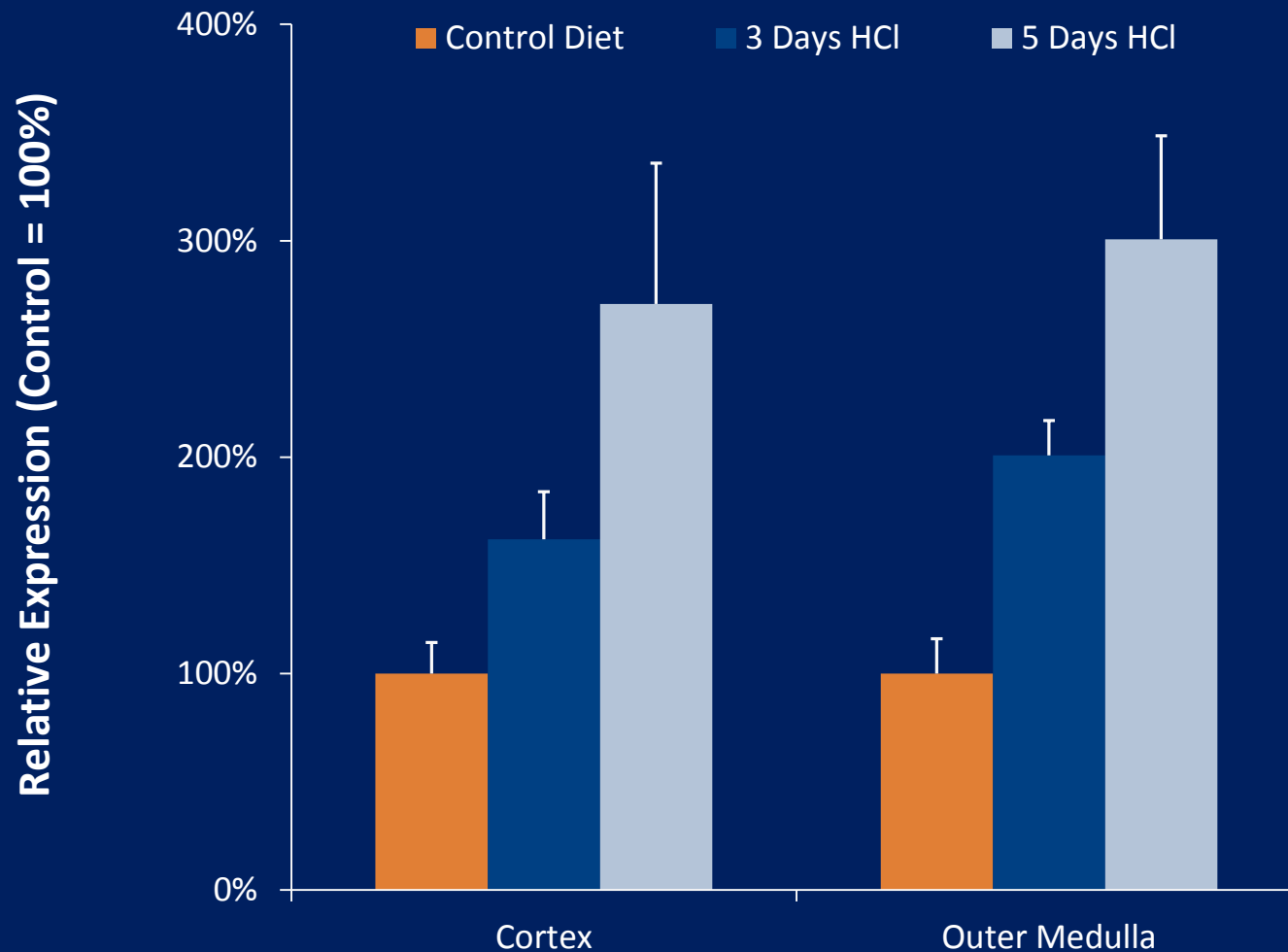


At least two modes of Rhcg regulation:

- Changes in steady-state protein expression
 - Post-translational regulation
- Trafficking to and from plasma membrane

Relative roles of each are cell-specific

Metabolic acidosis increases Rhbg expression



Conditions where Rhbg and/or Rhcg expression parallels ammonia transport

- Metabolic acidosis
 - Seshadri RM, et al, *AJP Renal* 290: F397-408, 2006.
 - Seshadri RM, et al, *AJP Renal* 290: F1443-52, 2006.
 - JM Bishop, et al. *AJP Renal* 299:F1067-77, 2010.
- Reduced renal mass
 - HY Kim, et al, *AJP Renal* 293:F1238-F1247, 2007.
- Ischemia-reperfusion injury
 - KH Han, et al, *AJP Renal* 293:F1342-F1354, 2007.
- Cyclosporine A-induced renal tubular acidosis
 - SW Lim, et al, *Nephron Exp Nephrology* 110:e49-58, 2008.
- Hypokalemia
 - KH Han, et al, *AJP Renal* 301:F823-F832, 2011
- Adaptive response to deletion of other acid-base transporters
 - Pendrin
 - ♦ Kim YH, et al, *AJP Renal* 289:F1262-F1272, 2005.
 - Collecting duct Rhcg
 - ♦ HW Lee, et al, *AJP Renal* 296:F1364-F1375, 2009.
 - Intercalated cell-specific Rhcg
 - ♦ HW Lee, et al, *AJP Renal* 299:F369-F379, 2010.
 - Intercalated cell-specific Rhbg
 - ♦ JM Bishop, et al, *AJP Renal* 299:F1065-F1077, 2010.

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Renal collecting duct NH_3 transport is both diffusive and saturable



Rh glycoproteins are present specifically in cells that transport NH_3

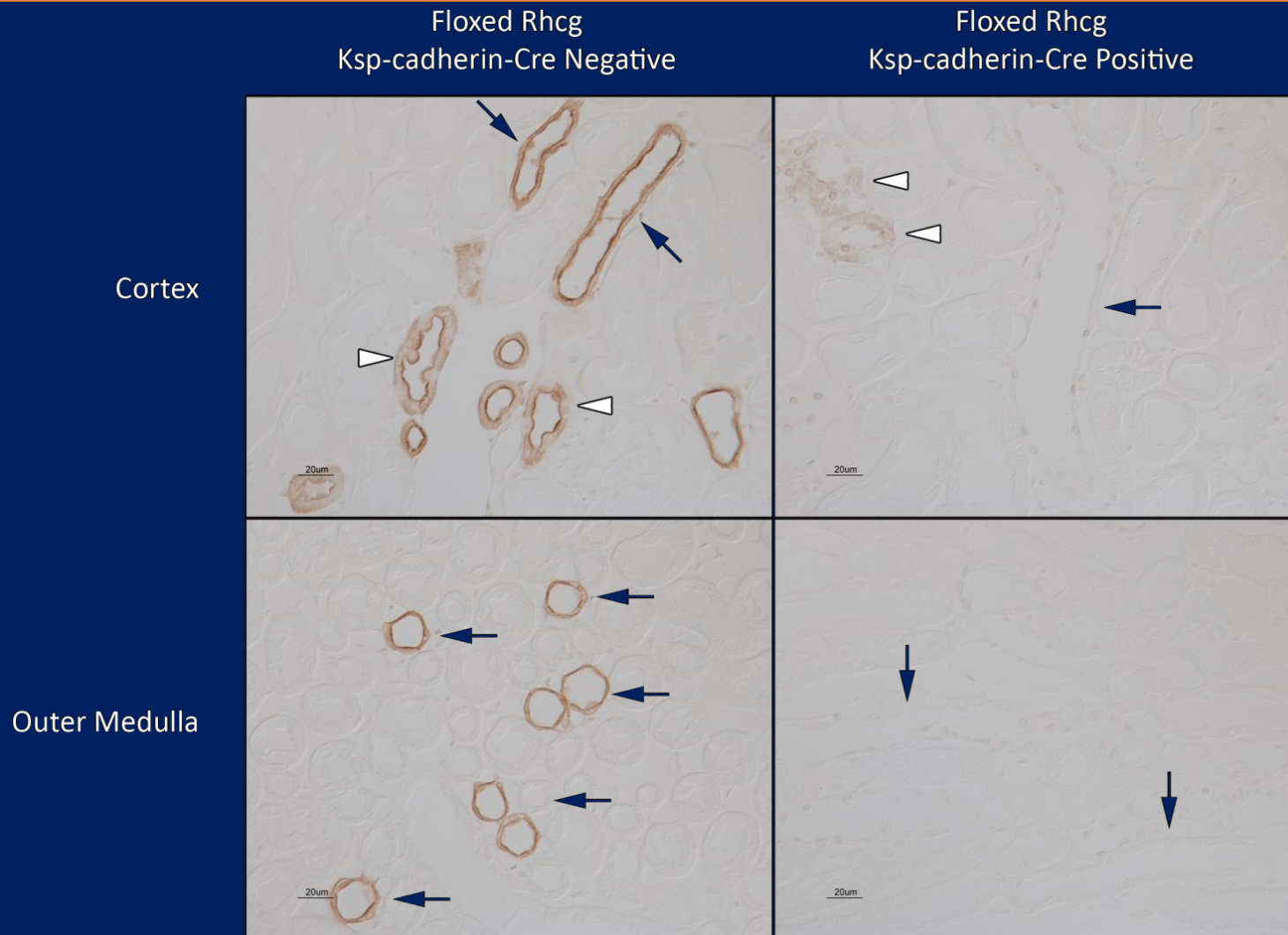


Rh glycoprotein expression parallels NH_3 gas transport

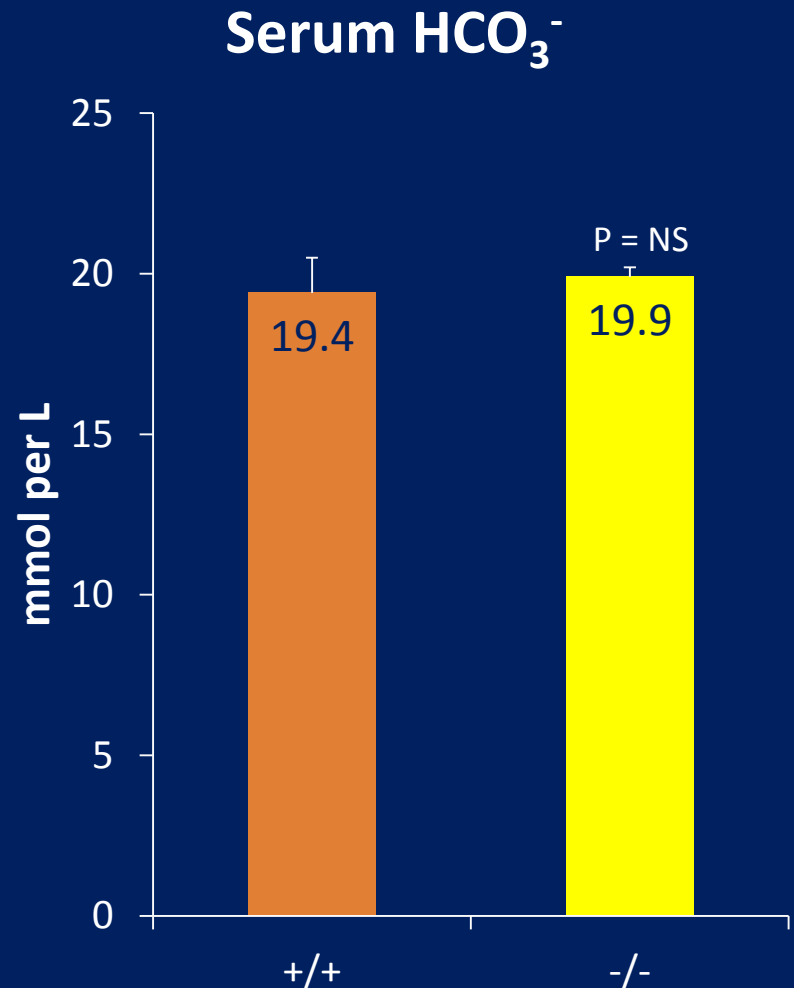
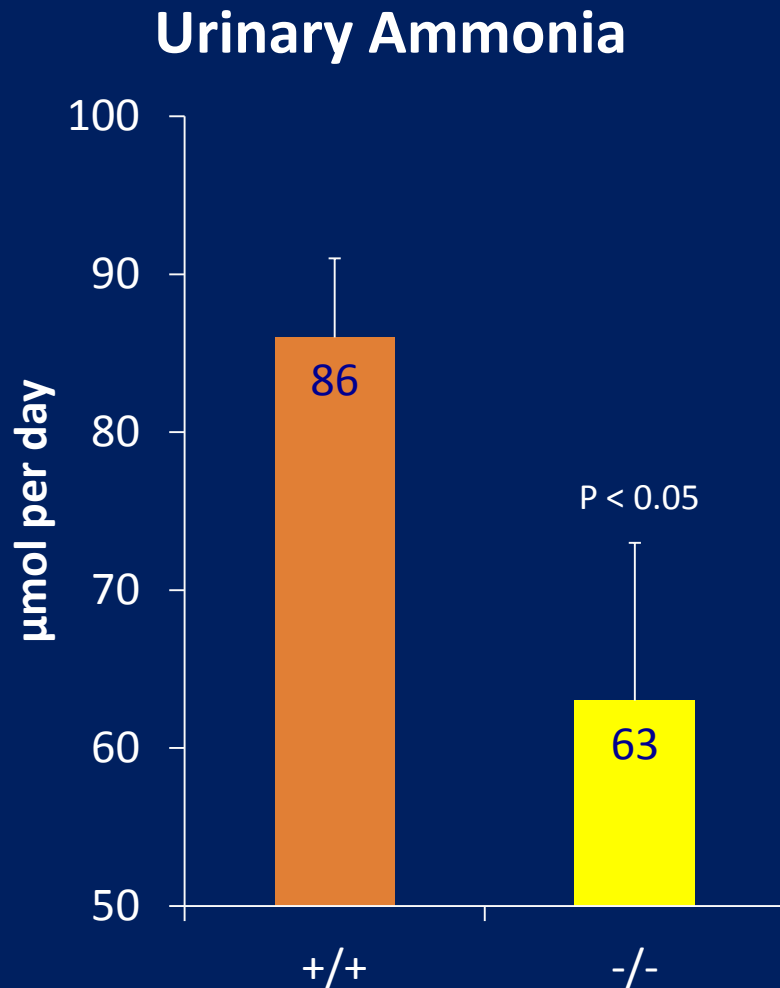


Does Rh glycoprotein inhibition alter NH_3 gas transport?

Collecting duct-specific Rhcg deletion

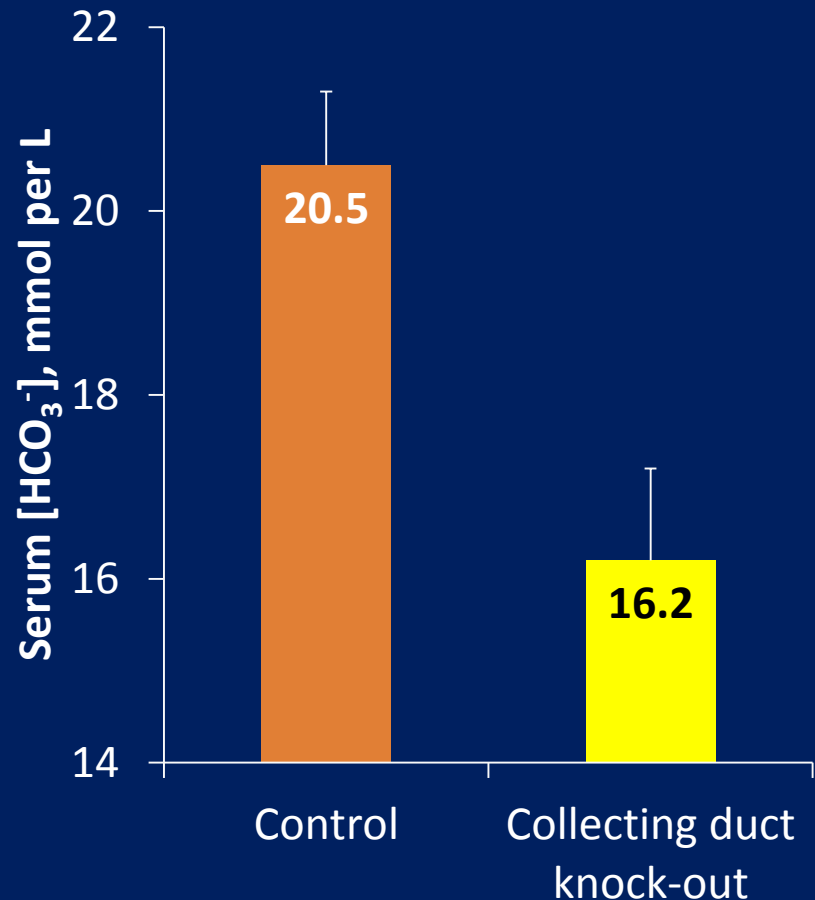


Collecting duct-specific Rhcg deletion - basal effects

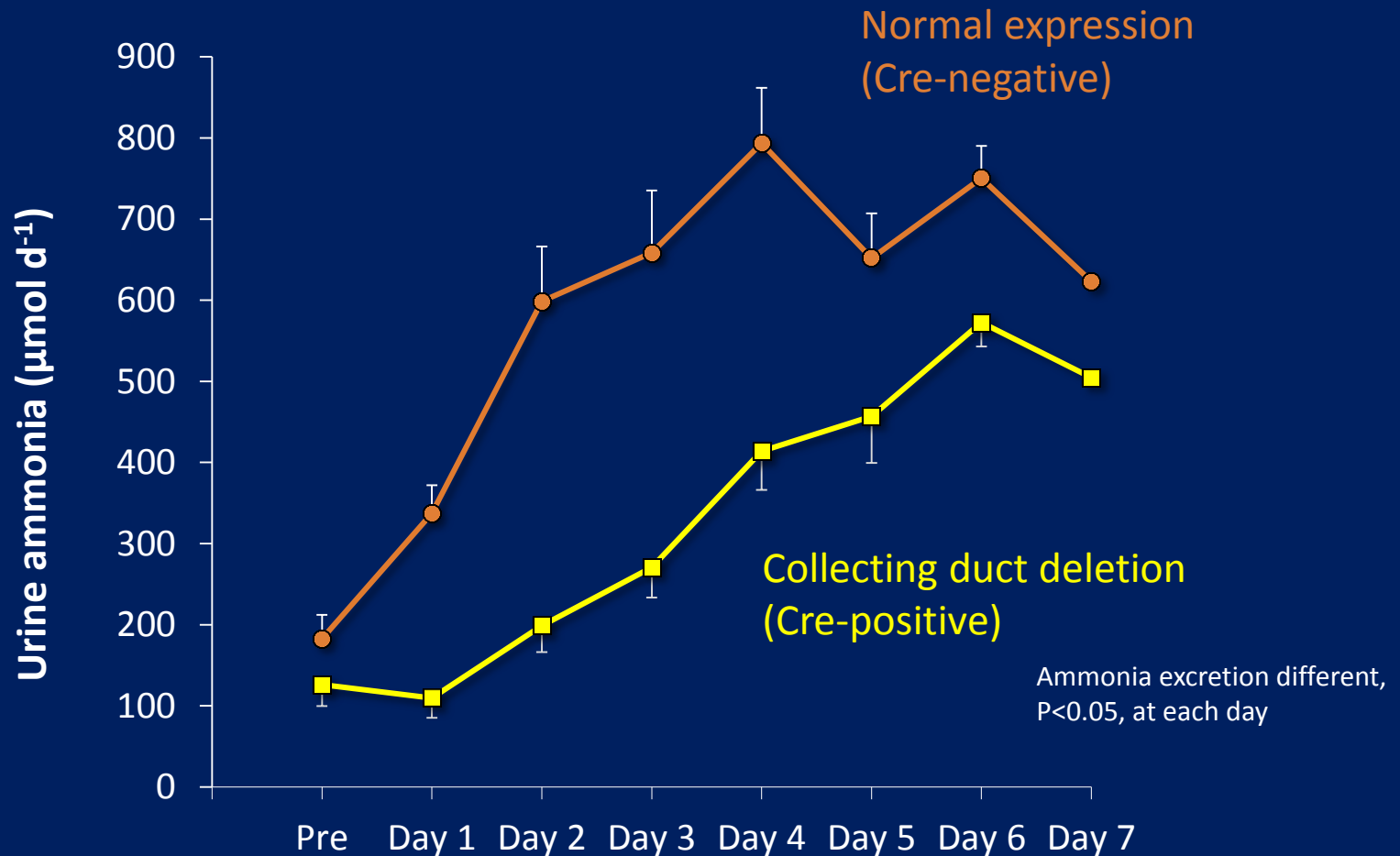


Collecting duct-specific Rhcg deletion - acid loading

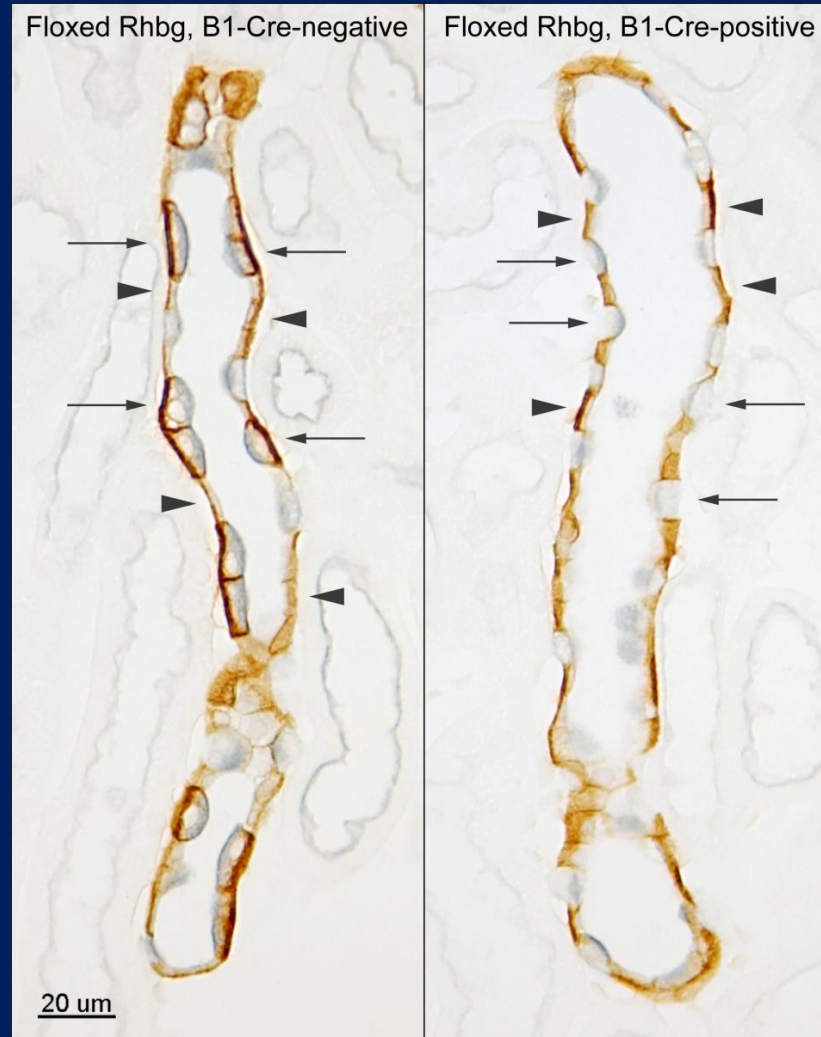
- Traditional
 - Add NH_4Cl to drinking water
 - Not well tolerated
 - ◆ Add glucose
 - Hard to quantify
- “New”
 - Add HCl directly to food
 - Use powdered food
 - Easily quantified



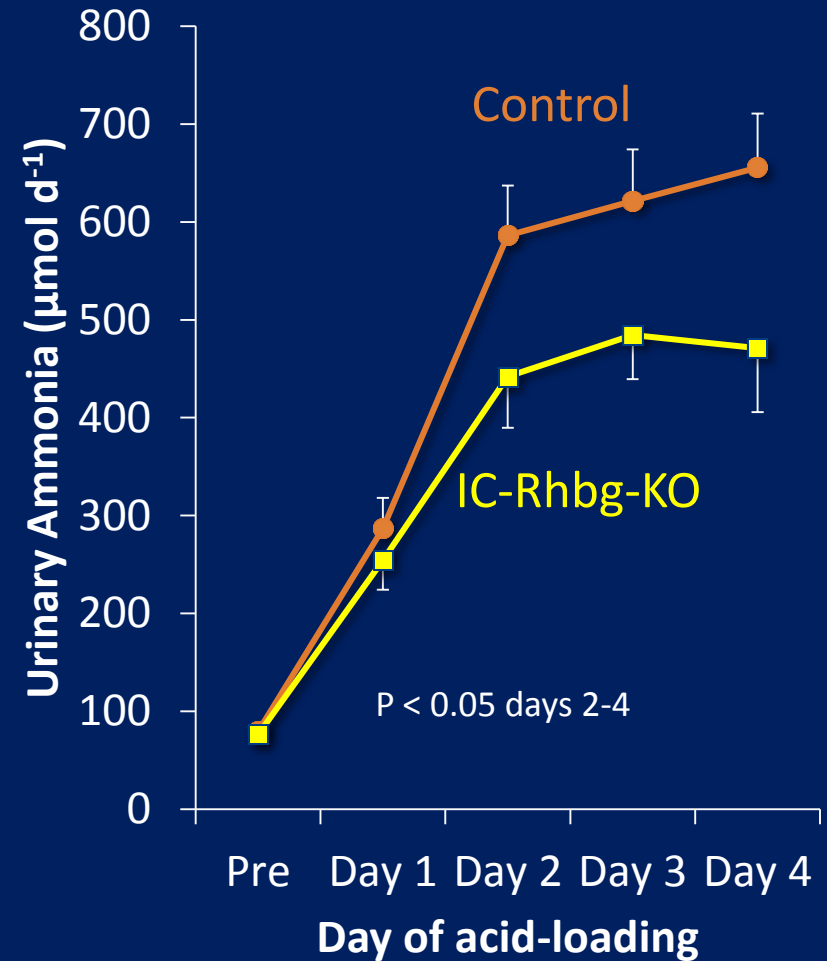
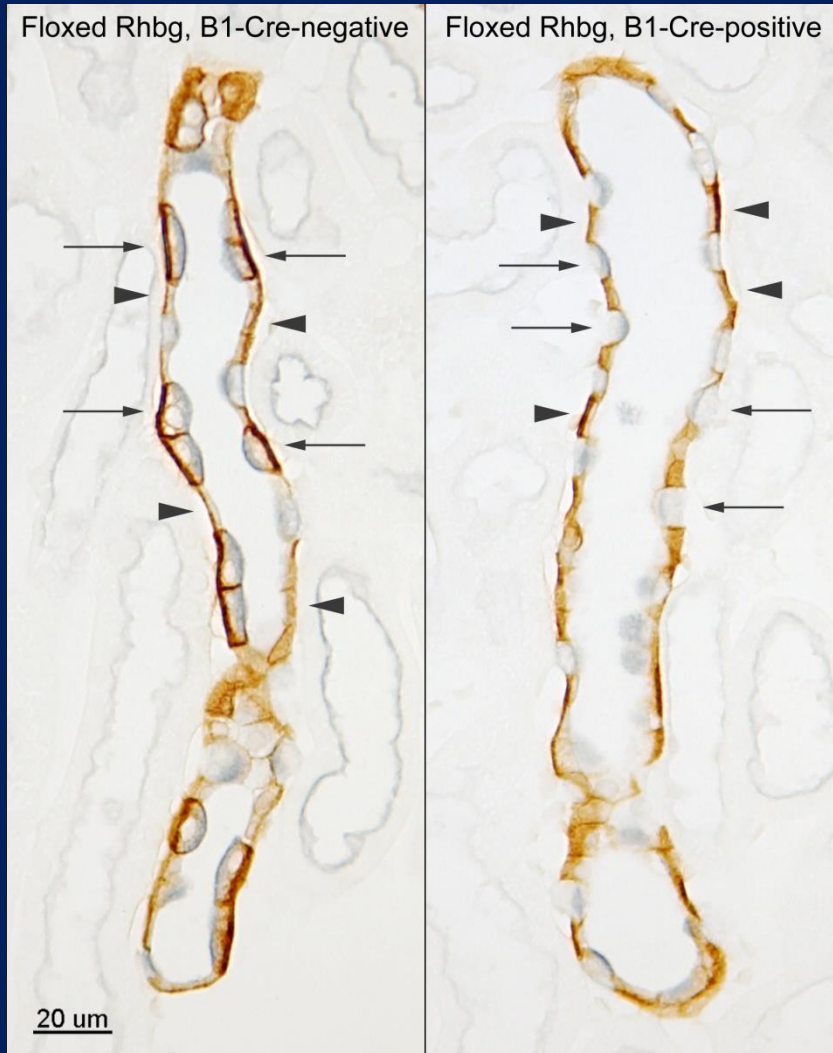
Collecting duct-specific Rhcg deletion - acid loading



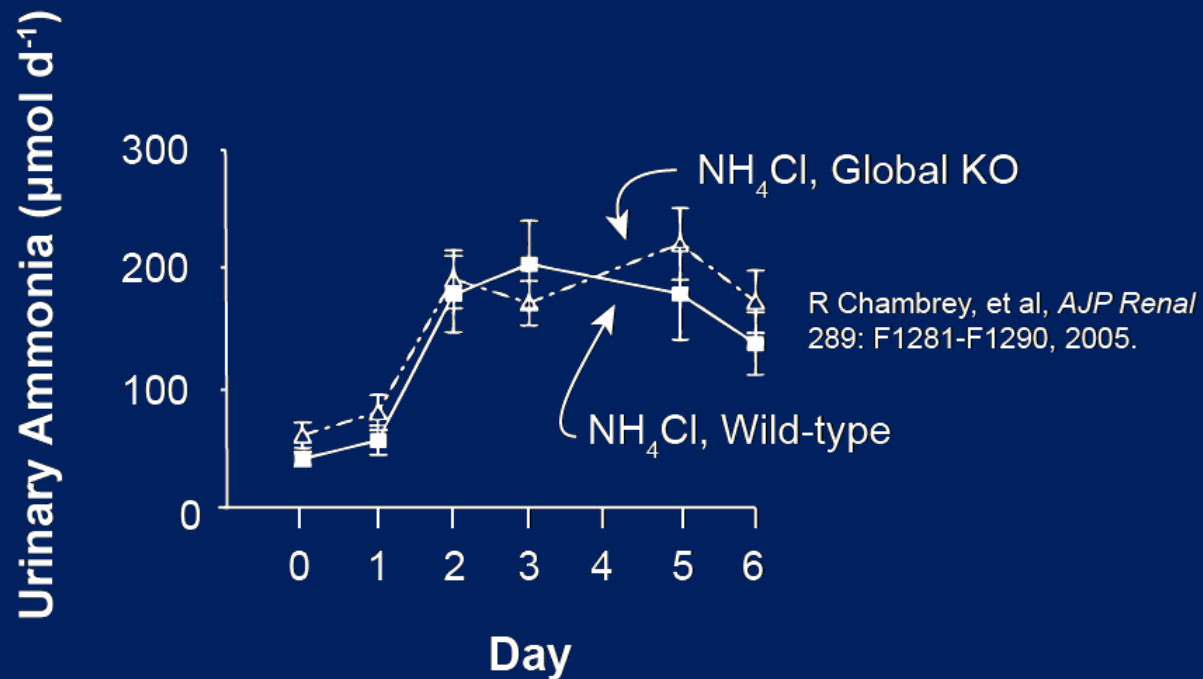
Development of intercalated cell-specific Rhbg knock-out mouse



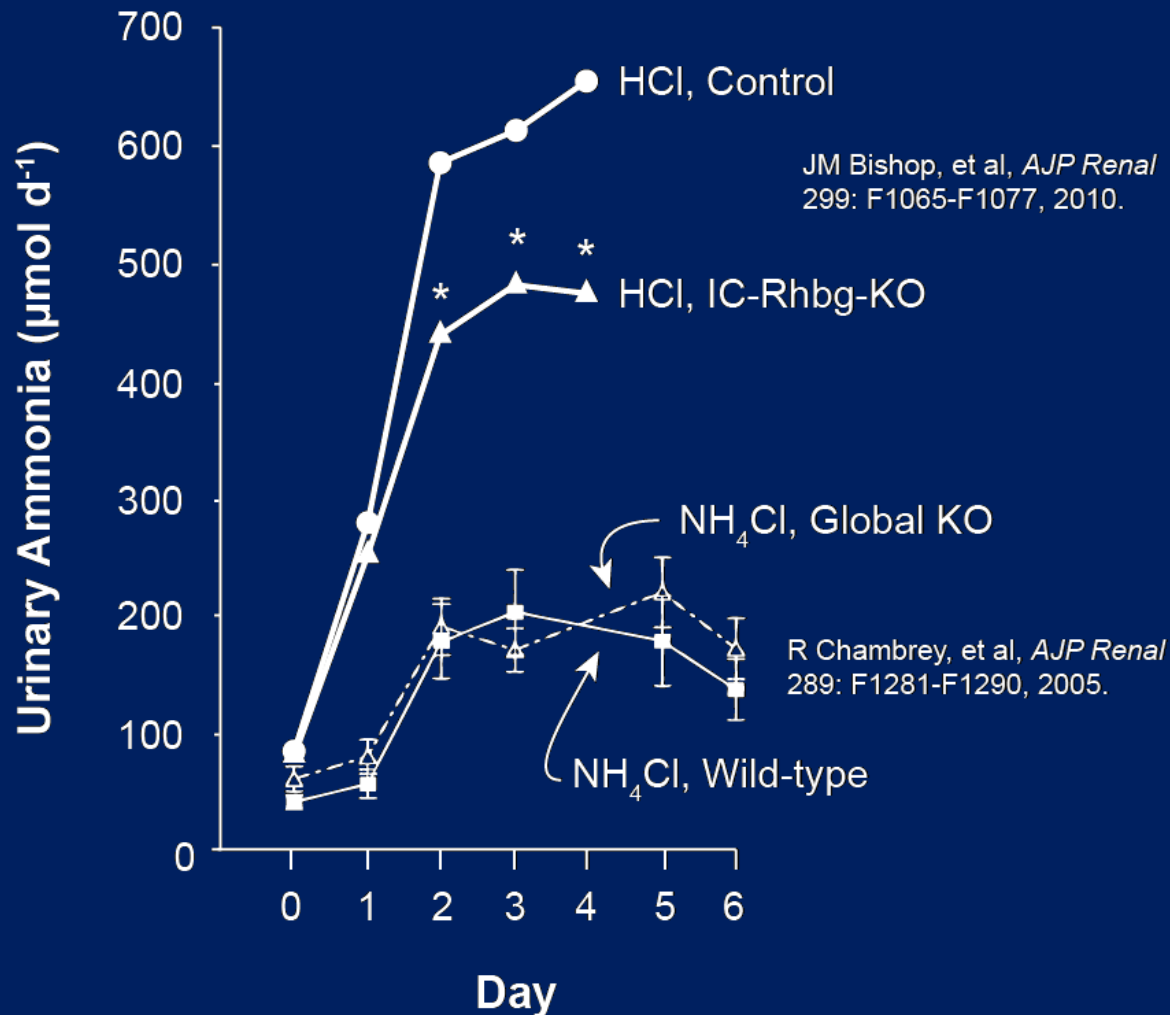
Effect of intercalated cell-specific Rhbg deletion on response to metabolic acidosis



Another Rhbg gene deletion study



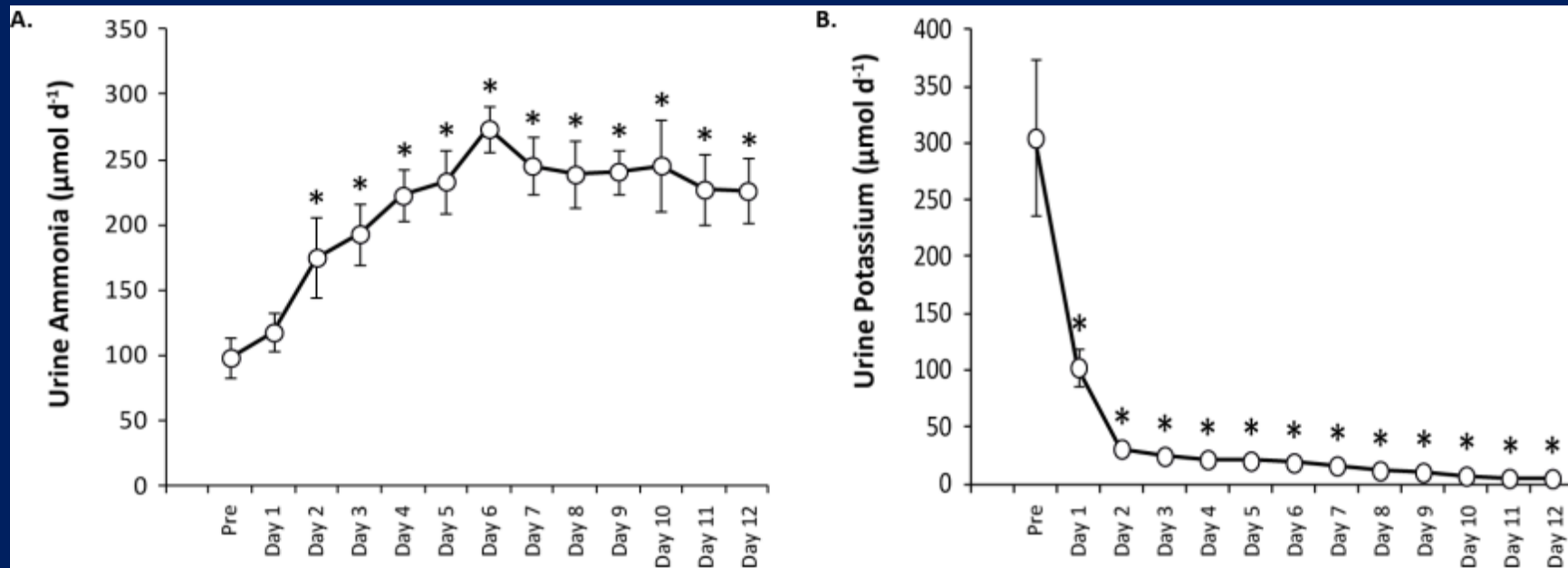
Comparison of the two Rhbg gene deletion studies



Does the role of Rhbg and Rhcg differ in different conditions?

- Hypokalemia
 - Increased urinary ammonia excretion
 - Urine alkalization
 - ◆ Increased urine acidification cannot be the primary driving force
 - Development of metabolic alkalosis

Effect of K⁺-free diet on mouse urinary electrolytes



Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

Renal collecting duct NH_3 transport is both diffusive and saturable



Rh glycoproteins are present specifically in cells that transport NH_3

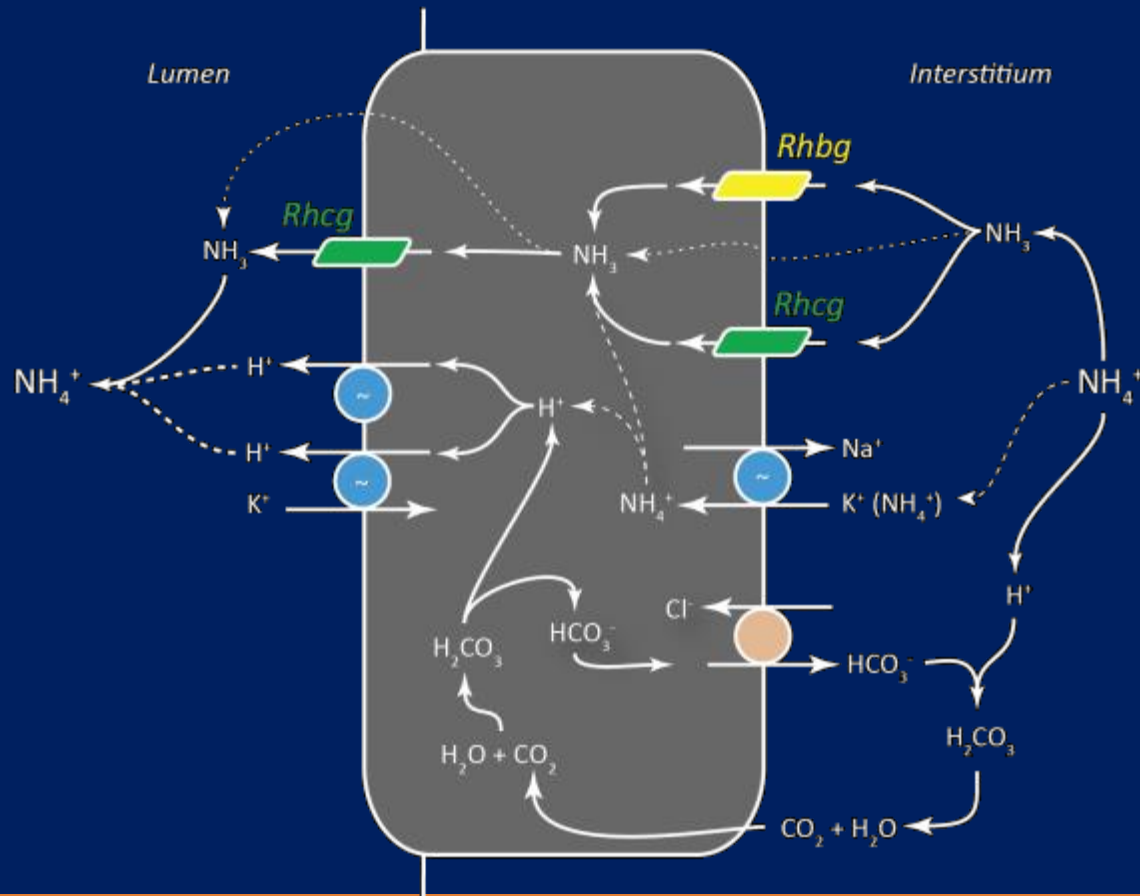


Rh glycoprotein expression parallels NH_3 gas transport



Rh glycoprotein gene deletion alter NH_3 gas transport

Rh glycoprotein-mediated NH_3 transport is central to renal ammonia metabolism and transport



'Role of Membrane Proteins in Oxygen Transport in Red Blood Cells '

R. Ryan Geyer, Ph.D.

PI: Walter F. Boron M.D., Ph.D.

Dept of Physiology & Biophysics

Case Western Reserve University School of Medicine

Cleveland, Ohio, USA

Gas Channel Workshop

September 7th, 2012 Cleveland, OH

Evidence for Gas Channels

The Boron Lab identified the first gas channel—the water channel AQP1— which exhibits permeability to CO₂.
Nakhoul et al, *AJP Cell* **274**, 1998

Cooper & Boron, *AJP Cell* **275**, 1998

DIDS—the anion transport inhibitor—not only reduces HCO₃⁻ permeability, but the CO₂ permeability in human RBCs.
Forster et al, *PNAS* **95**, 1998

Later it was shown that NH₃ also passes through AQP1.
Nakhoul et al, *AJP Renal* **281**, 2001

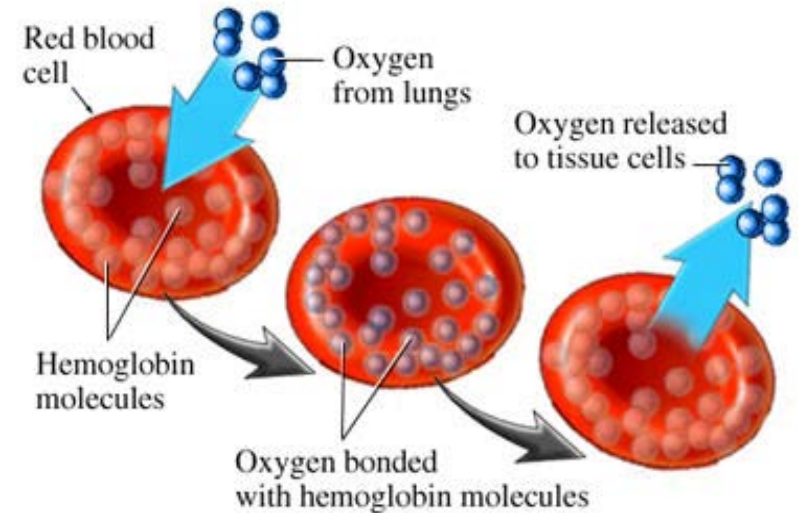
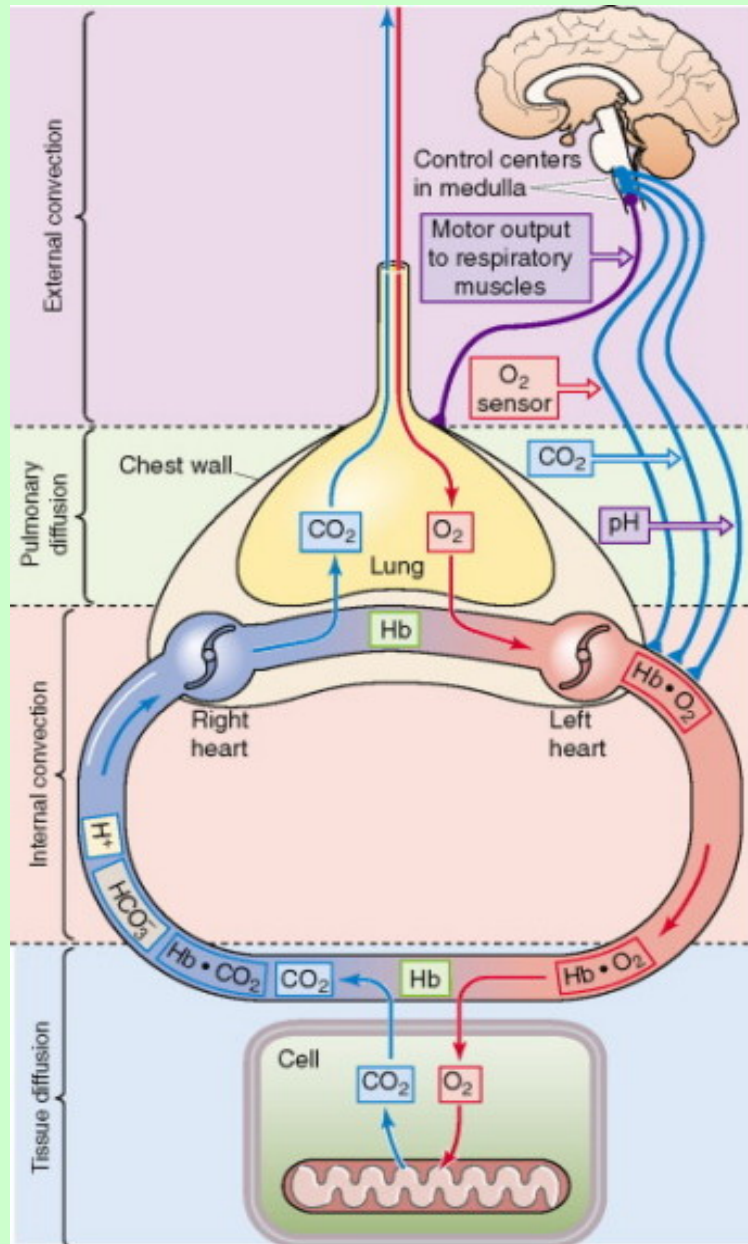
RhAG (a component of the Rh complex in human RBC) conducts NH₃.
Ripoche et al. *PNAS* **101**, 2004

Lipid vesicles containing AQP1 increased Nitric Oxide influx by about 300%.
Herrera et al. *Hypertension* **48**, 2006

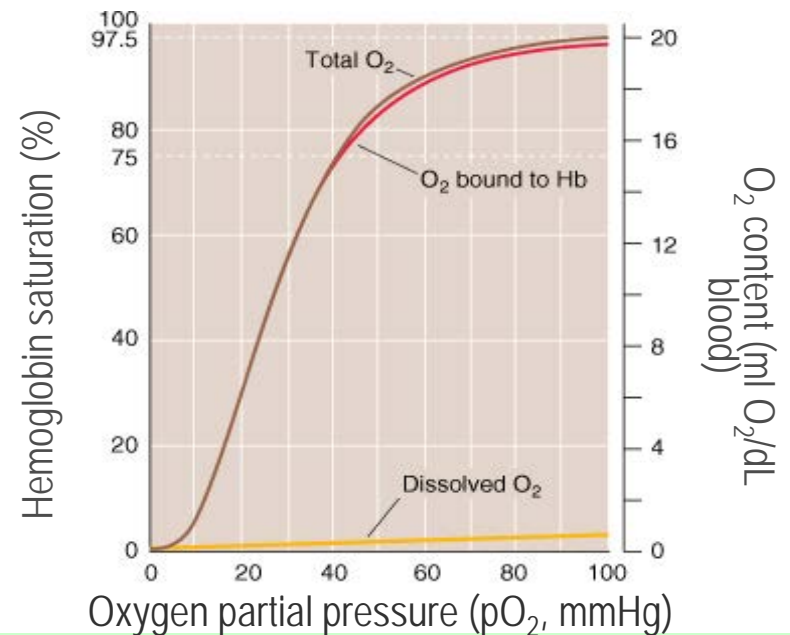
Endeward *et al.* found that the lipid of the RBC membrane has an extremely low permeability to CO₂.
Endeward et al, *FASEB J* **20**, 2006

Musa-Aziz & Boron showed for the first time that gas channels—like ion channels—can exhibit selectivity for one gas over another (CO₂ vs. NH₃).
Musa-Aziz et al, *PNAS* **106**, 2009

Gas Exchange



<http://www.infobarrel.com/media/image/96184.jpg>



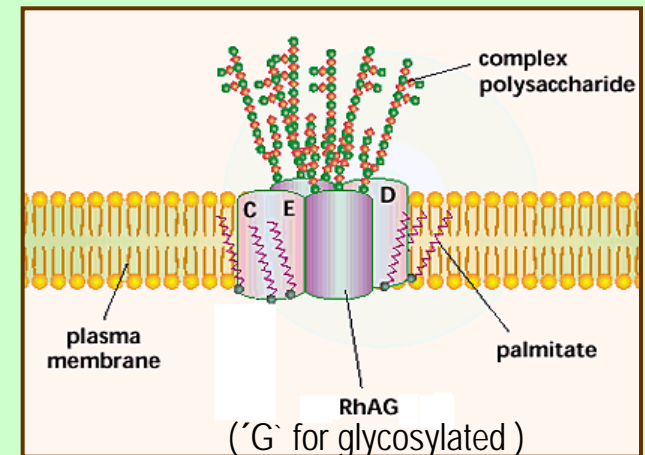
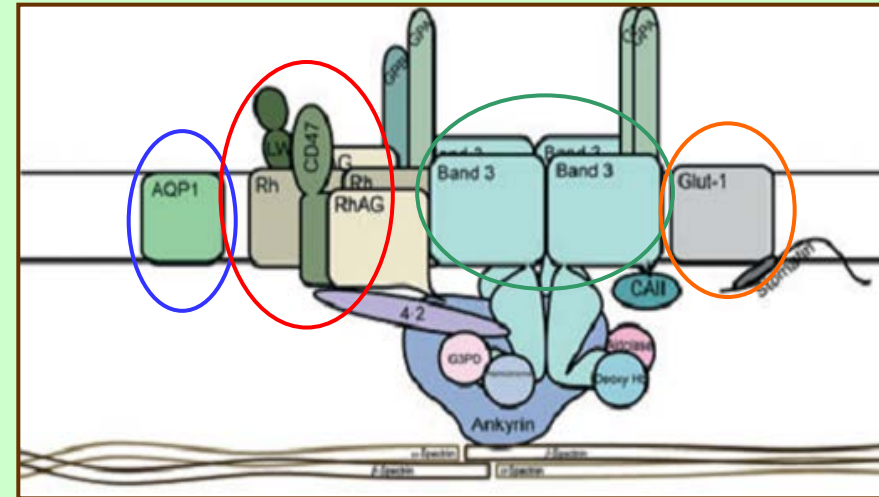
Significance

Due to the lipid and protein composition it is likely that these red blood cells (RBCs) have a low intrinsic permeability to gases.

Therefore, it would make physiological sense to have gas channel(s) to increase the O_2 flux, and that such a protein would be highly expressed in the RBC membrane.

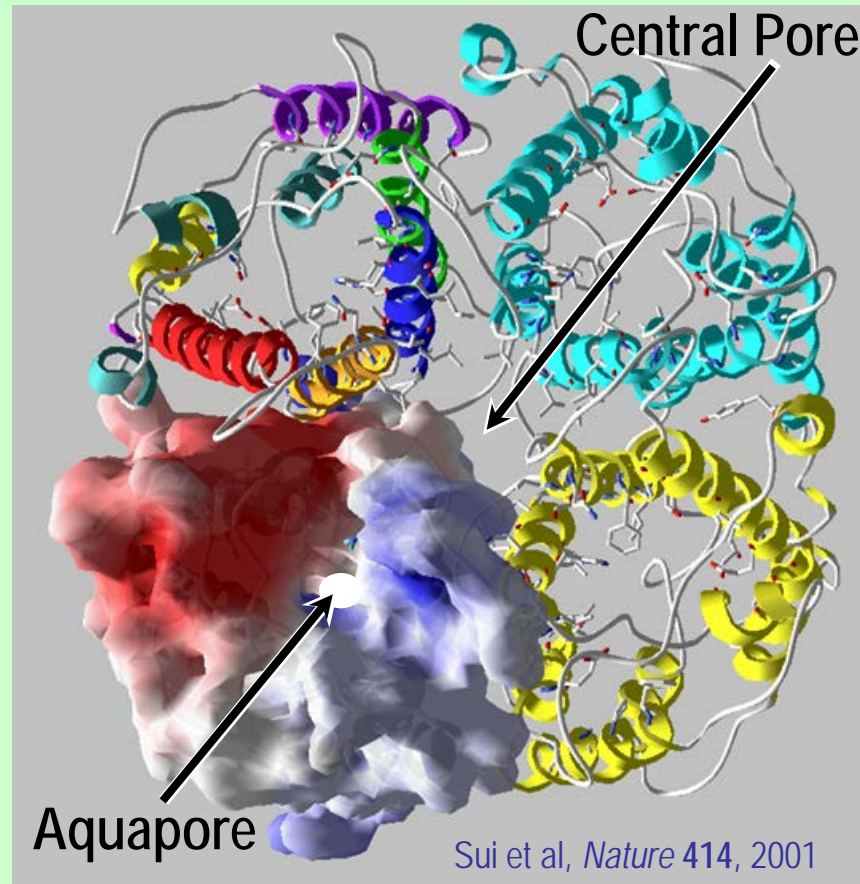
Key Membrane Proteins Present at High Levels in the RBC

- **AE1** (1 million copies per cell)
 - Cl/HCO₃⁻ co-transporter, Band 3
 - 25% of the cell membrane surface
 - Inhibited by DIDS
- **GLUT1** (humans), **GLUT4** (mouse) (600,000 copies per cell)
 - Glucose transporter
 - In mice there is a switch at about day 5
Montel-Hagan et al. Cell 132, 2008
 - Inhibited by pCMBS and phloretin
- **AQP1** (Aquaporin 1) (200,000 copies per cell)
 - H₂O, NH₃, and some CO₂ transport inhibited by pCMBS
 - Major CO₂ pathway inhibited by DIDS
- **Rh-complex** (Rhesus) (100,000 copies)
 - Transports NH₃
 - CO₂ transport blocked by DIDS
- **MCT-1** (Monocarboxylate Transporter 1) (80,000 copies per cell)
 - Inhibited by DIDS and pCMBS
- **UT-B** (Urea Transporter) (15,000 copies per cell)
 - Urea transport inhibited by pCMBS and phloretin
 - We have shown that UT-B can transport H₂O & NH₃, but not CO₂



All of these proteins form homo-oligomers of dimers, trimers, or tetramers, which could form additional pores.

AQP1 Crystal Structure

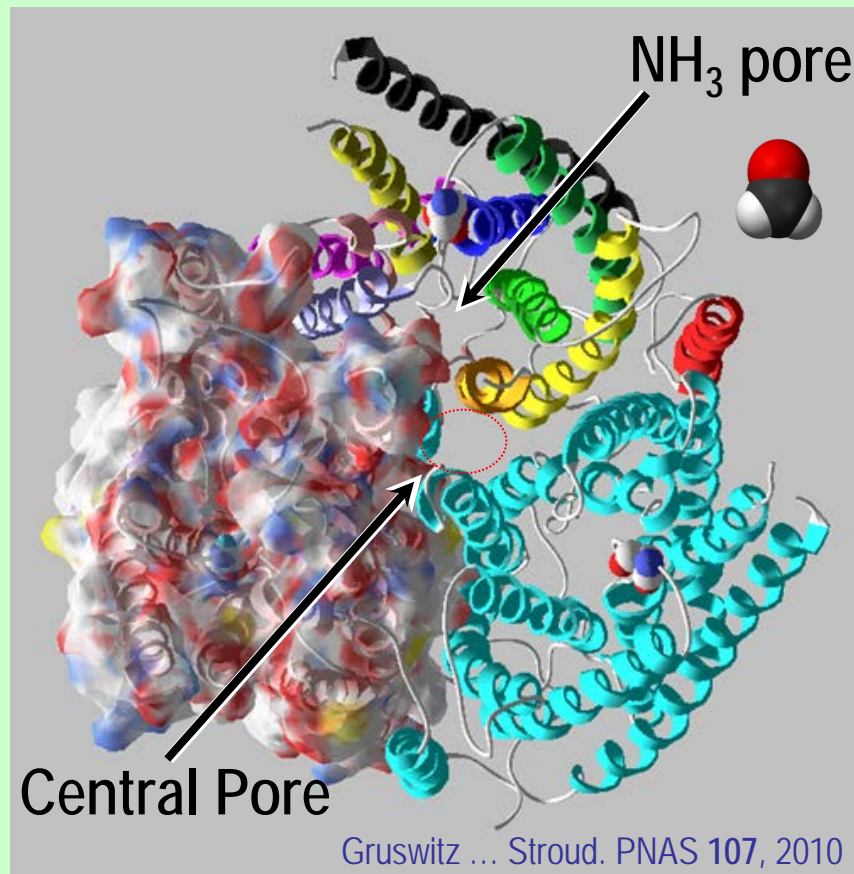


AQP1 is a **homotetrameric** protein with 4 water channels ... 'aquapore', which is lined by hydrophilic & hydrophobic residues.

There is an additional pore ...the 'central pore', which is lined by hydrophobic residues.

Our Laboratory showed that AQP1 can transport both CO_2 and NH_3 .

RhCG Crystal Structure

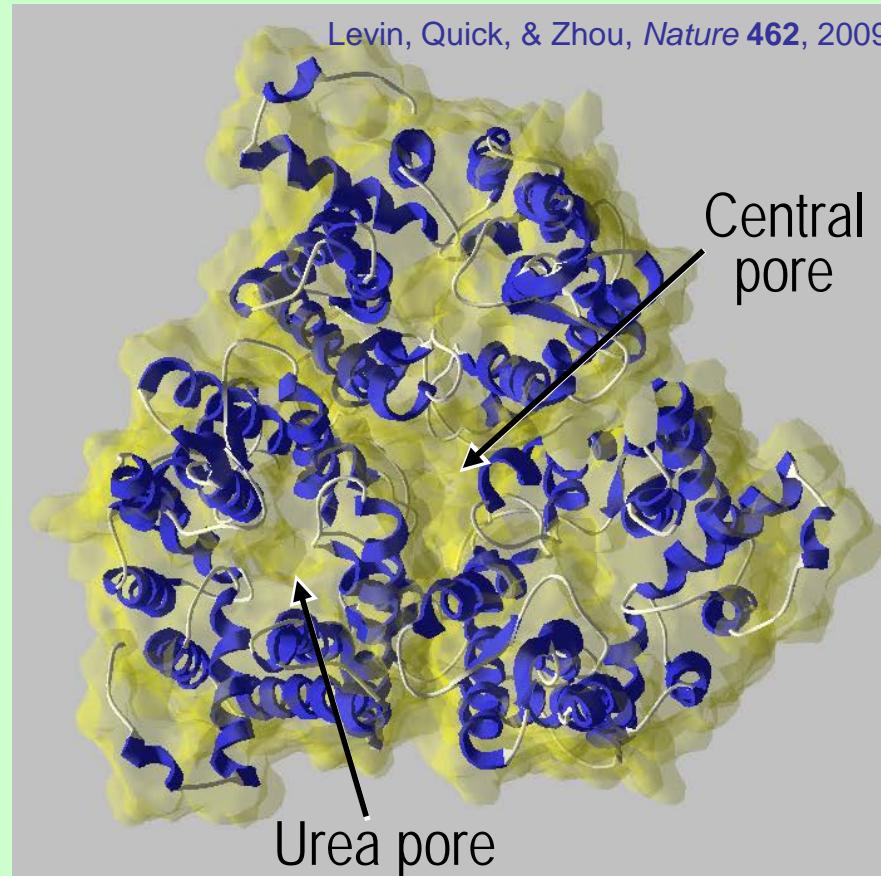


RhCG is a **homotrimeric** protein – each monomer contains 12 TMs and works as a functional ammonia pore ...

... playing an essential role in the secretion of NH₃ in the kidney, which is critical to systemic acid-base homeostasis.

Hydrophobic '**central pore**' is formed at the threefold axis of symmetry.

Urea Transporter Crystal Structure



The bacterial homolog Urea Transporter (UT-B) was crystallized as a homotrimer.

Each bundle of helices forms a monomeric urea channel.

Urea transport can be inhibited by phloretin, HgCl_2^{2+} , and pCMBS.

Our Questions ...

Can we quantitate the O₂ efflux and/or influx rate of intact wild-type RBCs?

If so, is it possible to determine the contribution of the CO₂ channels (AQP1 and Rh-complex) to the O₂ efflux?

Are there other RBC membrane proteins that could also contribute to the O₂ efflux?

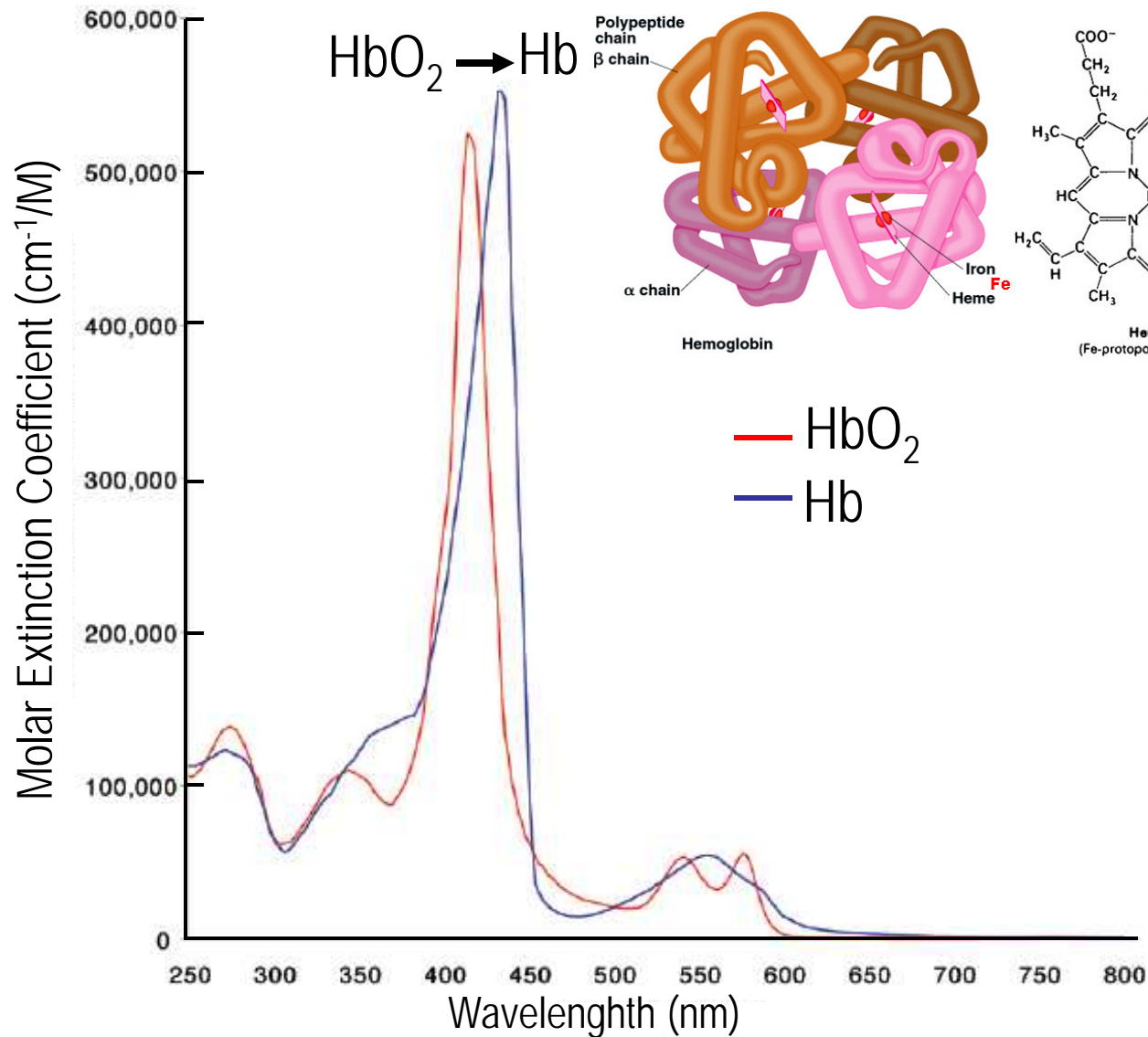
Can known inhibitors of transporters block O₂ efflux from the RBC?

pCMBS – mercurial agent that covalently reacts with cysteine thiol groups. Blocks water transport via AQPs and a variety of other transport processes.

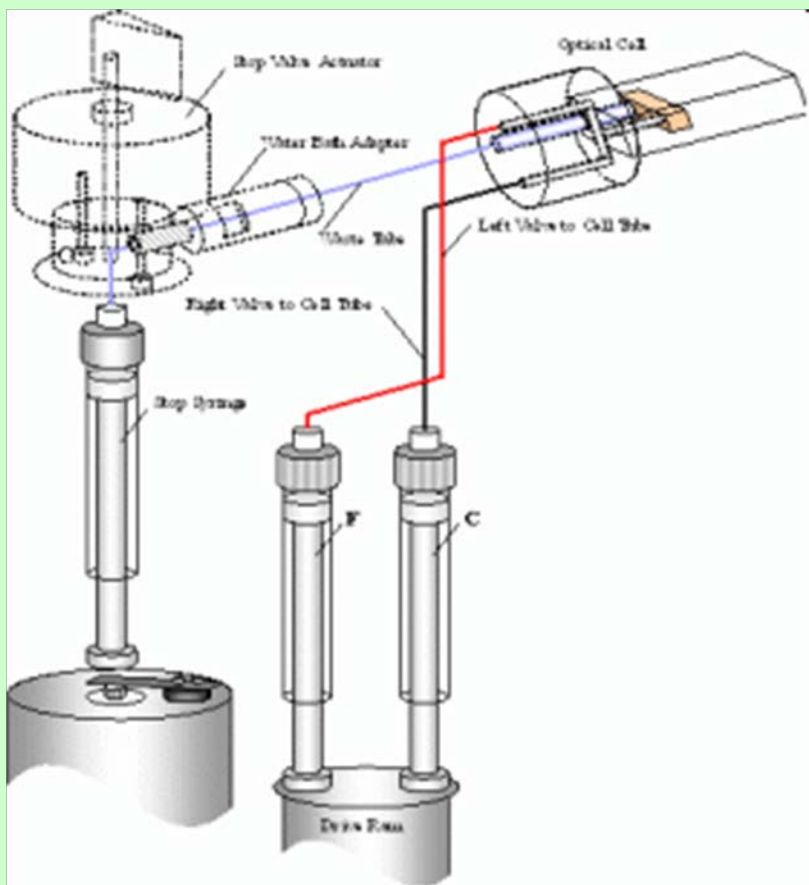
DIDS – amino-reactive agent known to inhibit the anion transporter (AE1 or Band 3) activity. The interaction can be reversible and irreversible (covalent). Also shown to reduce the CO₂ permeability of AQP1 and RhAG.

Phloretin – known to inhibit the glucose and urea transporters.

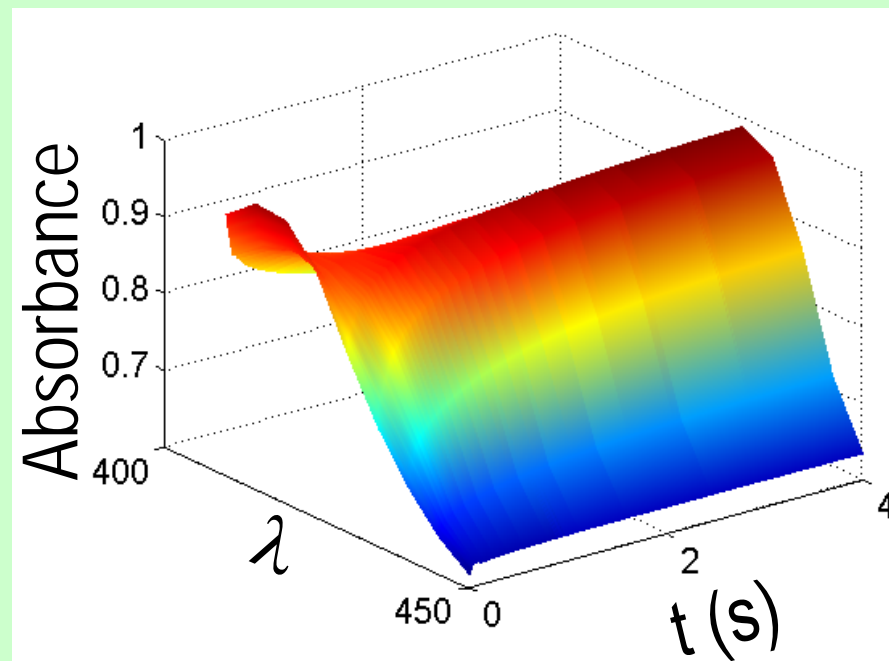
Hemoglobin Absorbance Changes



Stopped Flow Technique



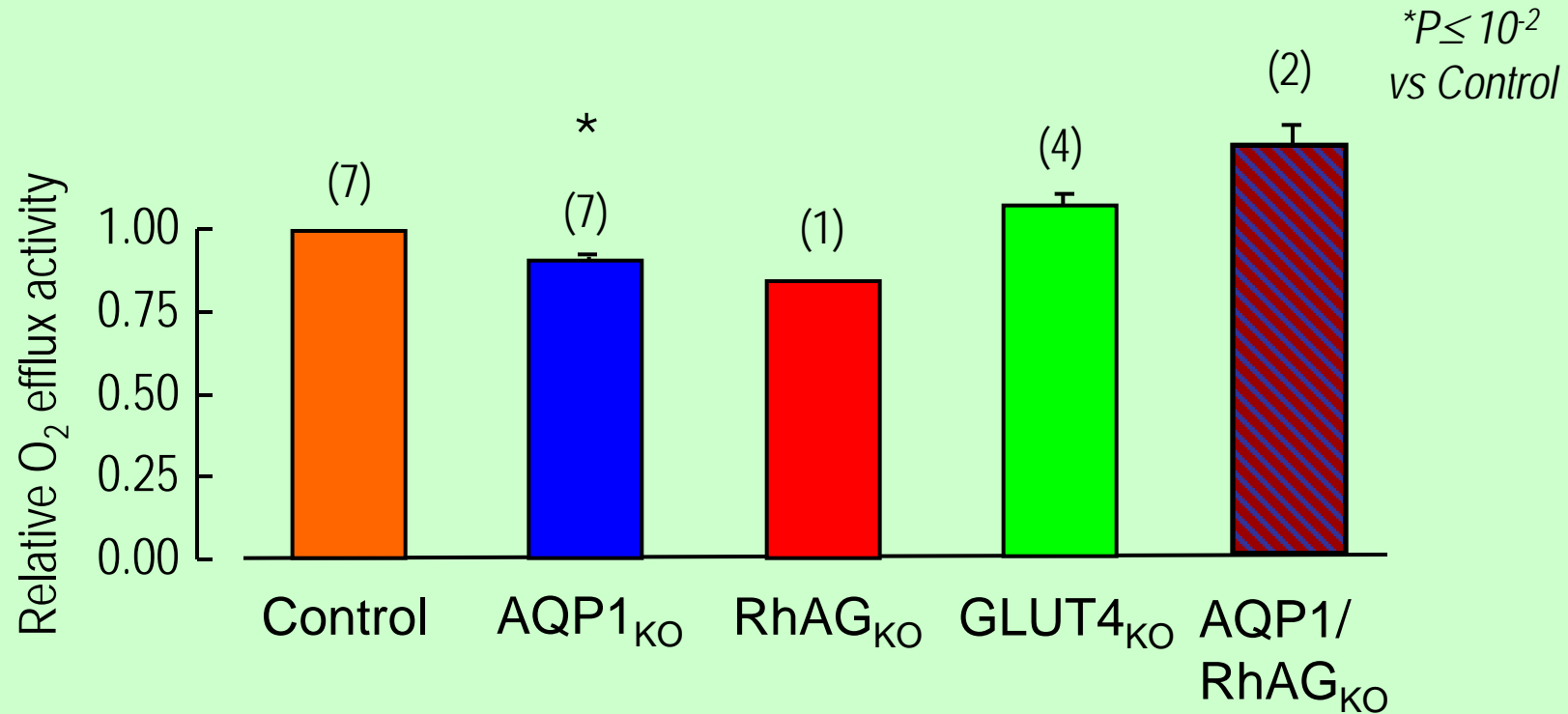
Rapidly mixes two solutions
(on a millisecond timescale)



Data from multiple wavelengths (λ) can be collected and compiled into a 3-D graph (Abs. vs. Time vs. λ).

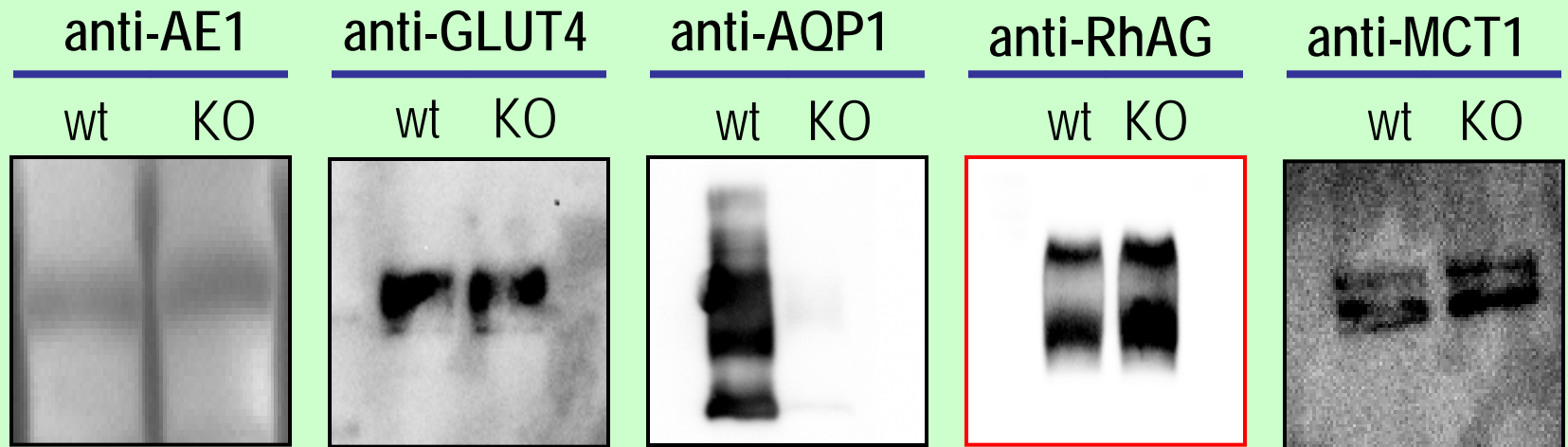
From this data we can calculate the rate of the chemical reaction.

Effect of knocking out AQP1, RhAG, GLUT4, and AQP1/RhAG on O₂ efflux in mouse RBCs

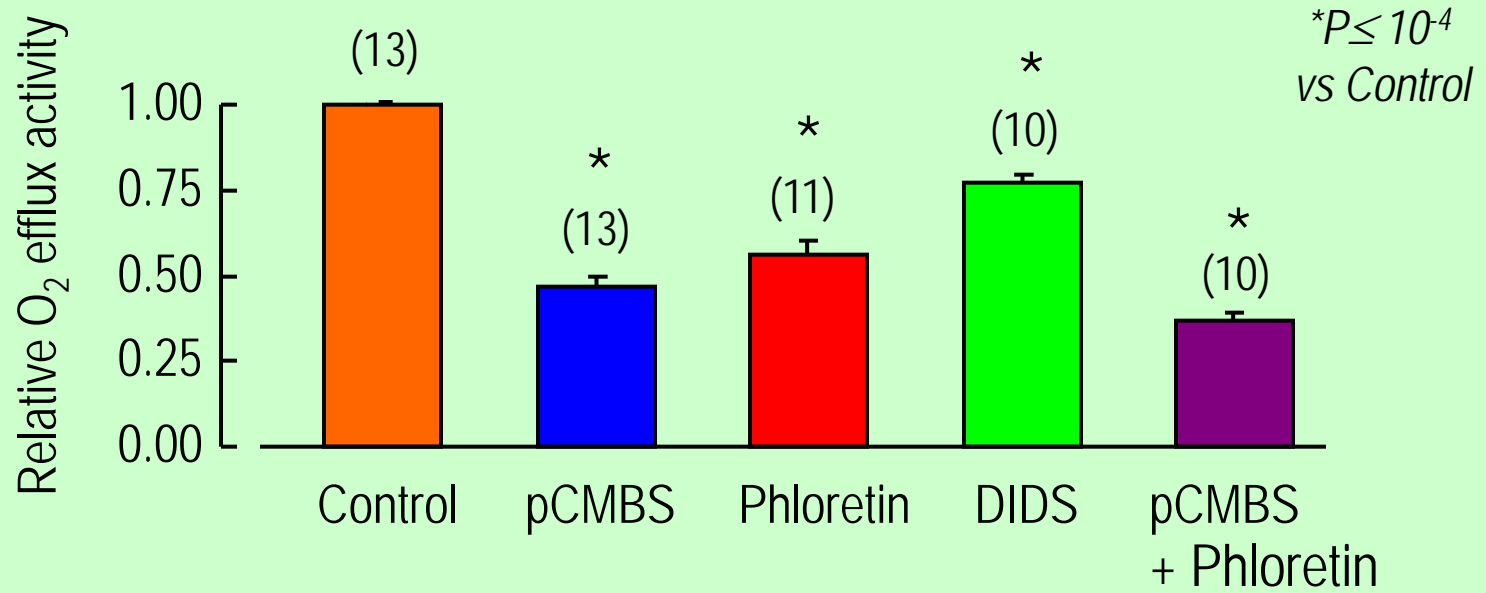


The major CO₂ channels of the RBC—AQP1 and RhAG—also function as modest O₂ channels.

Western Blot Analysis of Membrane Proteins from wild-type and AQP1-KO



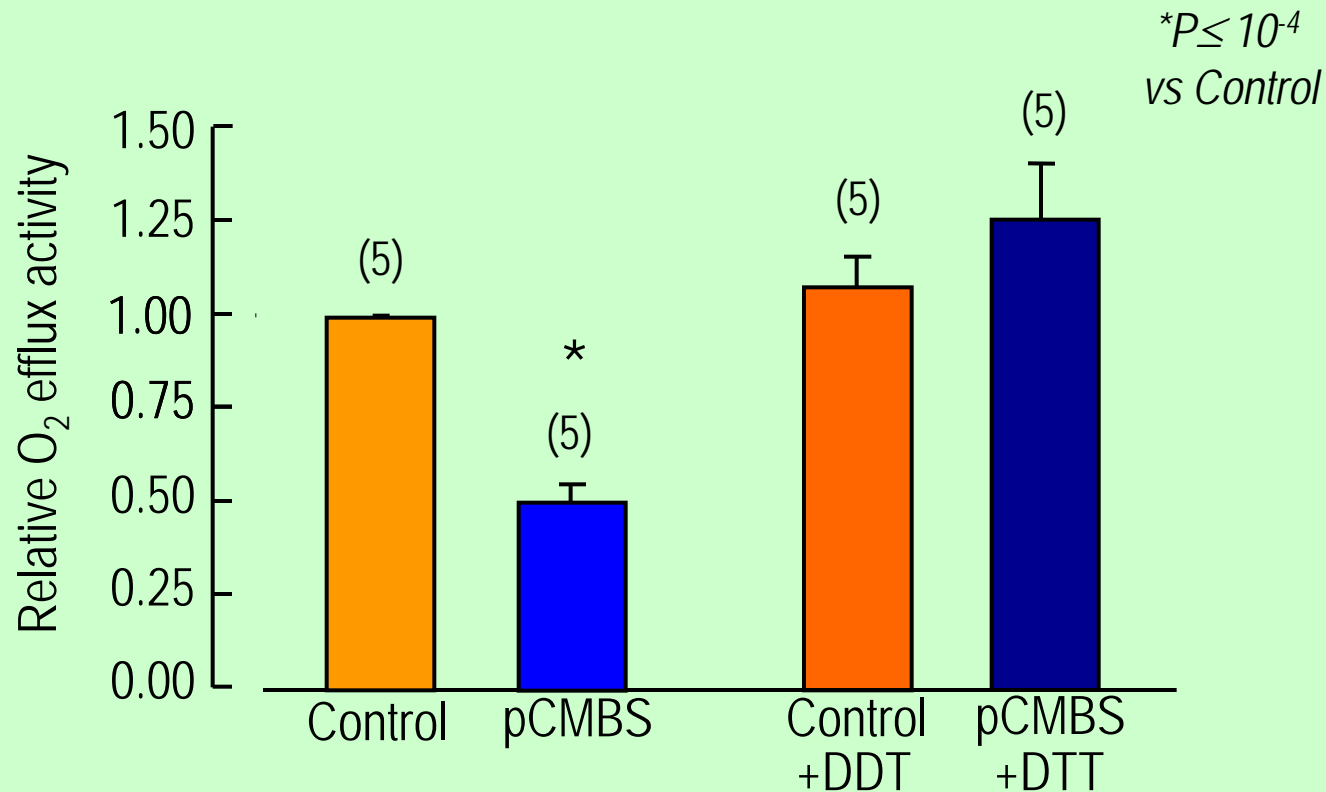
Effect of pCMBS, Phloretin, or DIDS on Oxygen Efflux from Mouse Red Blood Cells



- pCMBS reduces the O₂ efflux by 50%.
- Phloretin reduces the O₂ efflux by 40%.
- DIDS reduces the O₂ efflux by 25%.
- pCMBS + Phloretin reduce the O₂ efflux by 70%.

Inhibitor efficacy: pCMBS + phloretin > pCMBS > phloretin > DIDS

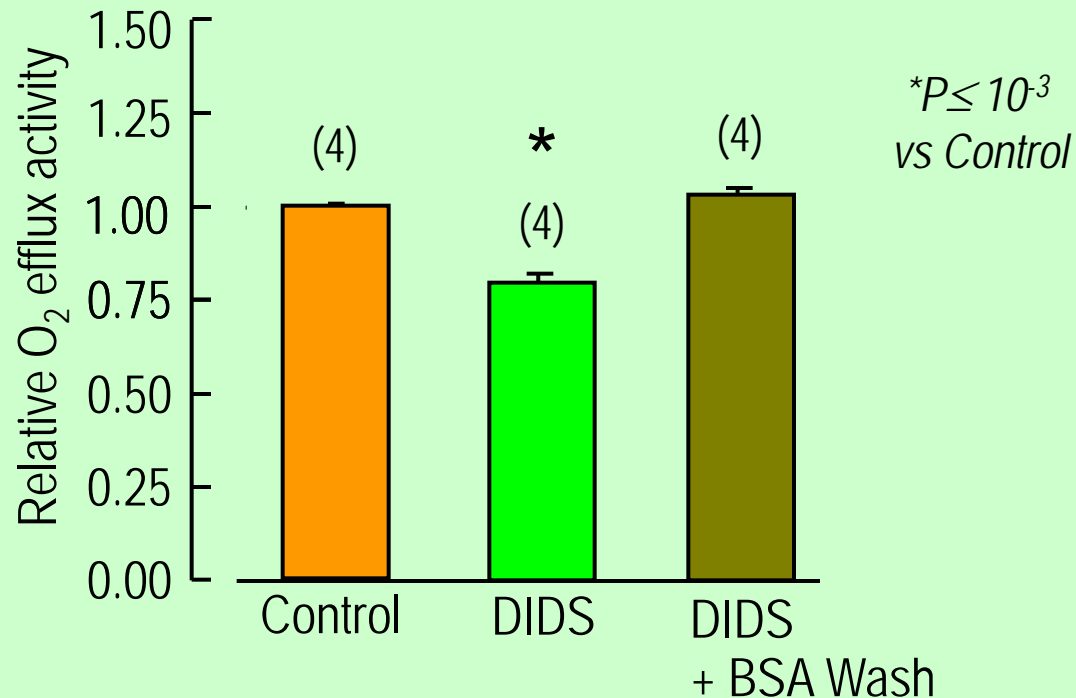
pCMBS Reversibility



As shown previously, pCMBS reduces the O₂ efflux rate by ~50%.

The inhibitory effect of pCMBS can be reversed with the addition of the reducing agent DTT.

DIDS Reversibility



The inhibition of O₂ efflux observed with DIDS appears to be non-covalent and reversible, the inhibition can be reversed when the RBCs are washed with 0.2% Bovine Serum Albumin (BSA).

Conclusions

- The data suggest that the DIDS-sensitive CO₂ channels (AQP1 and RhAG) of the RBC appear to modestly transport O₂ out of the cell.

... because this transport process is not greatly effected by DIDS the O₂ must utilize an alternative pathway(s).

- Knockouts of GLUT4 and AQP1/RhAG have O₂ efflux rates greater (10-25%) than the wild-type controls.
- Inhibitor efficacy:

pCMBS + phloretin > pCMBS > phloretin > DIDS

... because the pCMBS/Phloretin inhibition was not totally additive, O₂ transport likely occurs by two or more channels.

- There must be a pathway (channel) that is sensitive to both pCMBS and phloretin and another pathway that is insensitive to both inhibitors.

Future Directions

- Compile inhibitor profiles for the knockout mice.
- Perform western blots on RBCs from knockout mice.
- Develop assays for monitoring nitric oxide (NO) and hydrogen sulfide (H₂S) transport in red blood cells.
- Investigate the effect of hypoxia on protein expression (XQ) and O₂ transport activity (RRG).
- Investigate the effect of adding and removing cholesterol from the RBC membranes on O₂ transport activity.

Acknowledgments

Principal Investigator

Walter F. Boron, M.D., Ph.D.

Collaborator

Raif Musa-Aziz, Ph.D. (Univ. of Sao Paulo)

Animal Technician

Thomas Radford (CWRU)



Talks & Posters

Geyer RR, MD Parker, N Burton, WF Boron, AM Toye, & R Musa-Aziz. Relative CO₂/NH₃ permeabilities of human RhAG, RhBG, and RhCG. Experimental Biology, Washington, DC, *FASEB J* 25:1040.4, 2011.

Geyer RR, R Musa-Aziz, & WF Boron. Evidence that DIDS crosslinks Aquaporin 1 monomers. Experimental Biology, Washington, DC, *FASEB J* 25:1039.26, 2011.

Musa-Aziz R, **RR Geyer** & WF Boron. Relative CO₂/NH₃ permeabilities of several members of the mammalian Aquaporin family: bAQP0, hAQP1, hAQP2, rAQP3, rAQP4-M1, rAQP4-M23, and hAQP8. Experimental Biology, Washington, DC, *FASEB J* 25:1040.5, 2011.

Geyer RR & WF Boron. Gas transport through channels. Undersea & Hyperbaric Medicine Society Scientific Meeting in conjunction with The Office of Naval Research, Fort Worth, TX, June 15-18, 2011.

Geyer RR, R Musa-Aziz, & WF Boron. Movement of NH₃ through Human Urea Transporter B (UT-B)—a new member of gas channels. ASN Kidney Week, 2011.

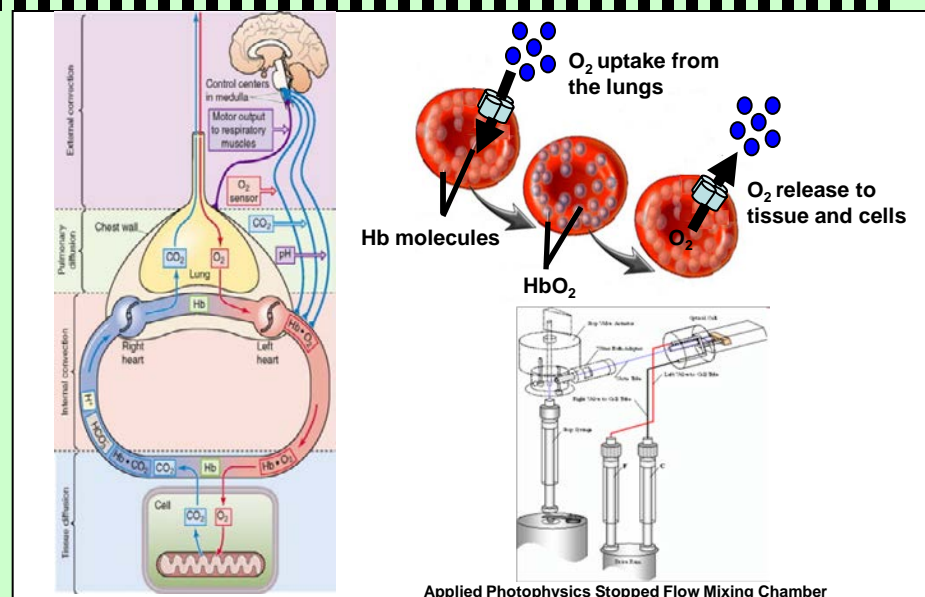
Musa-Aziz R, **RR Geyer**, X Qin, & WF Boron. The CO₂/NH₃ selectivities and inhibitor sensitivities of mammalian Aquaporins. ASN Kidney Week, 2011.

Geyer RR & WF Boron. Role of Membrane Proteins in Oxygen Transport in Red Blood Cells. International Workshop on Membrane Transport of Small Solutes, Strobl, Austria, June, 2012.

Background: Red blood cells (RBCs) function to carry oxygen (O_2) to tissues and transport carbon dioxide (CO_2) away from tissues. The traditional view had been that these gases dissolve in the membrane and diffuse into or out of the cell. Our laboratory and others have shown that RBC membrane proteins can function as gas channels for the transport of CO_2 and/or NH_3 . It is our hypothesis that membrane proteins can also facilitate the transport of O_2 across the RBC membrane. This research could represent a major paradigm shift, and totally reorganize our thinking of how O_2 crosses cell membranes.

Naval and Scientific Benefits: If we understand the molecular mechanism of gas transport—we could design pharmacological agents that—by inhibiting or activating gas channels—can prevent or treat **decompression illness** and **O_2 toxicity**.

Objectives: (1.) To quantitate O_2 efflux of intact RBCs using stopped-flow absorbance spectroscopy. **(2.)** Determine the contribution of the CO_2 channels (AQP1 and RhAG) to the O_2 efflux, as well as other highly expressed RBC membrane proteins (AE1, GLUT4, MCT1, and UT-B). **(3.)** Assess the effect on O_2 efflux rate when wild-type RBCs have been treated with compounds known to inhibit: H_2O permeability (pCMBS), glucose and urea transport (phloretin), and CO_2 transport (DIDS).



FY12 Accomplishments, Discoveries, & Inventions

- Determined O_2 efflux rate of intact, wild-type RBCs.
- Completed inhibitor profile of O_2 efflux from intact RBCs.
- Investigated O_2 efflux rate of intact RBCs from AQP1-null, RhAG null, and GLUT-4 null mice.

FY13 Goals

- Quantitate O_2 efflux rate of intact RBCs from AE1 null, GLUT-4 null, and UT-B null mice.
- Investigate the transport of nitric oxide (NO) and hydrogen sulfide (H_2S) in intact RBCs.

Principle Investigator: Dr. Walter F. Boron, 216-368-3400
walter.boron@case.edu

Gas Channel Workshop

Structure determinants for CO₂ transport of human aquaporin5

Xue Qin

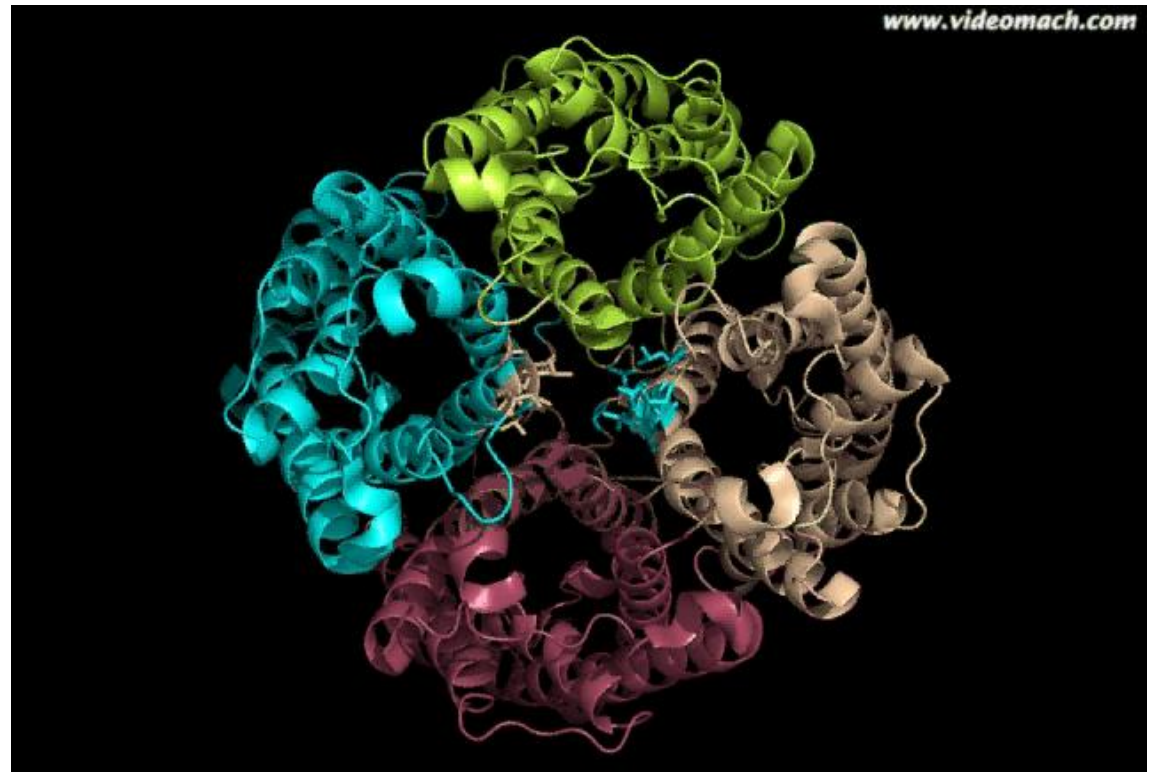
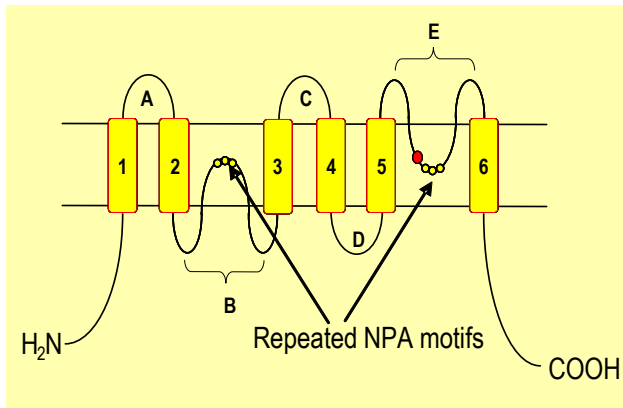
PI: Walter F Boron

Department of Physiology and Biophysics
Case Western Reserve University

Background

Aquaporin 5 is a water channel highly expressed in salivary glands, eye, lung and trachea.

Aquaporins are composed of 6 transmembrane domains, with N- and C- terminus on the cytoplasmic side of the membrane.



Background

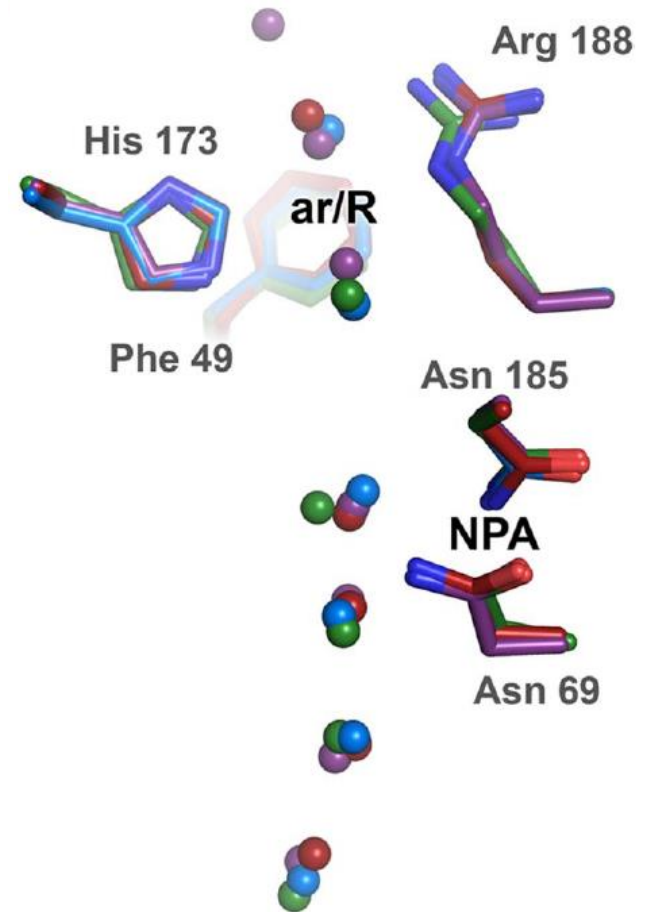
H₂O permeability

2 filter regions is important for H₂O transport

- Selectivity filter: ar/R region
- NPA region

CO₂ permeability

Not well defined

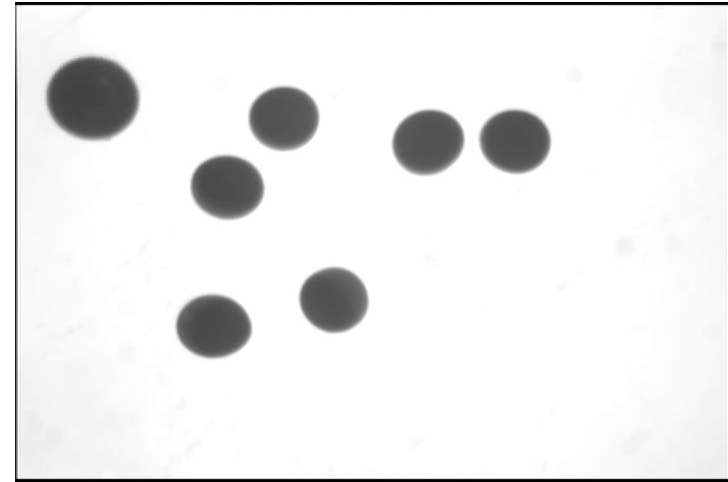


Horsefield et al, 2008 PNAS

Methods

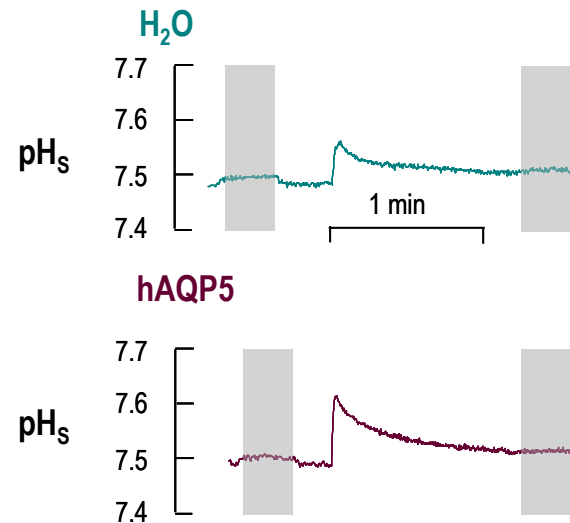
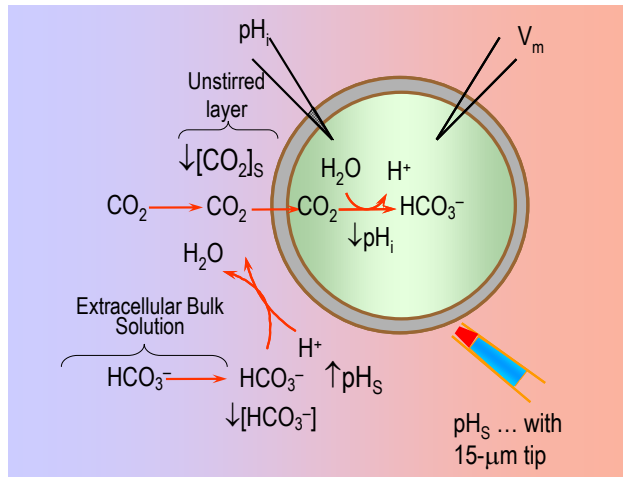
H_2O permeability-- P_f

Volumetric assay to measure osmotic water permeability
How fast the volume of oocytes change with time



CO_2 permeability-- ΔpH_s

Microelectrode to measure pH on the surface of the oocytes

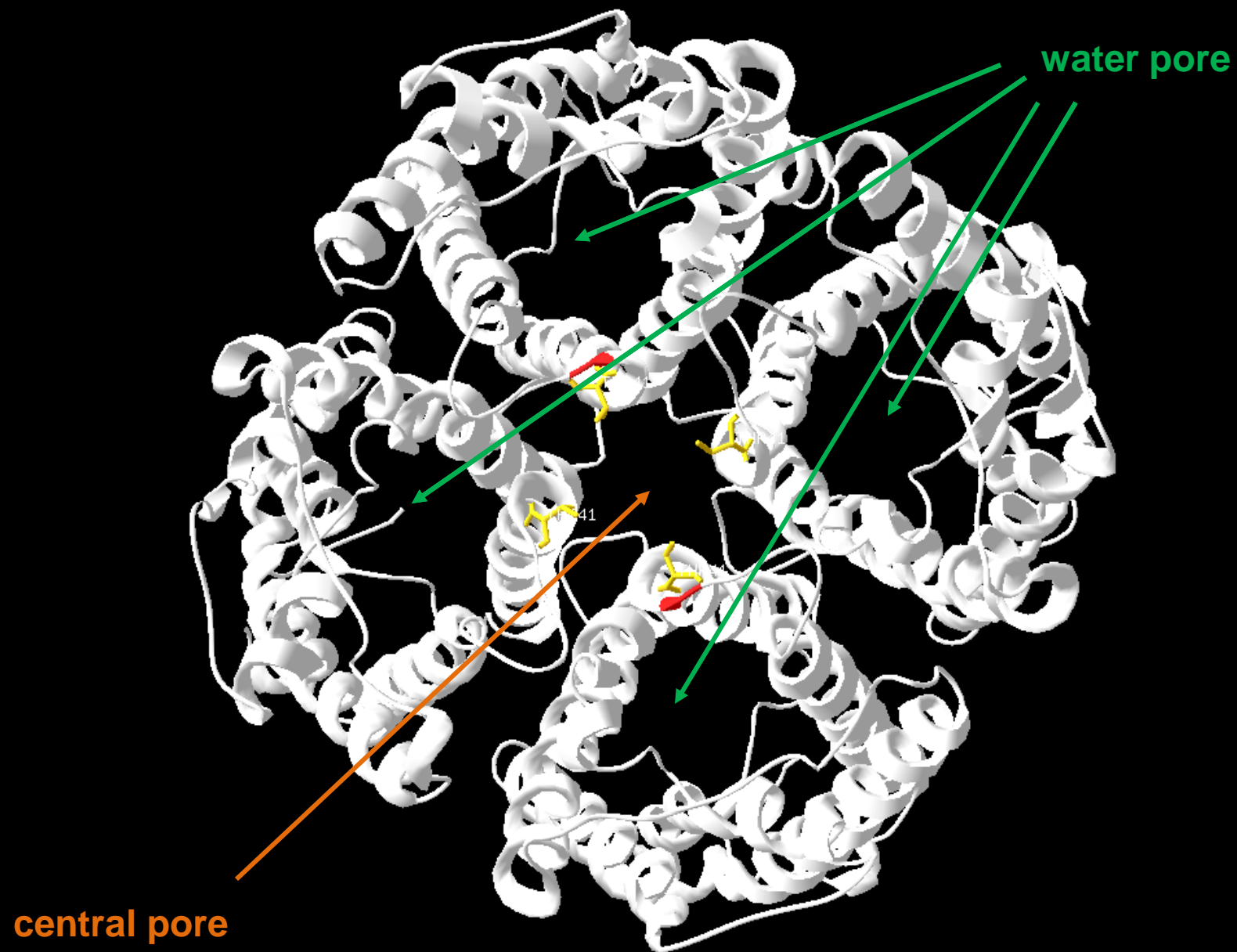


Outline

- I Amino acids at the mouth of the central pore
- II Amino acids lining the central pore

Outline

- I Amino acids at the mouth of the central pore
- II Amino acids lining the central pore



Exploring gas permeability of cellular membranes and membrane channels with molecular dynamics

Yi Wang ^a, Jordi Cohen ^{a,d}, Walter F. Boron ^b, Klaus Schulten ^{a,d}, Emad Tajkhorshid ^{a,c,*}

^a *Theoretical and Computational Biophysics Group, Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

^b *Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520, USA*

^c *Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

^d *Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

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Available online 4 January 2007

Abstract

Aquaporins are a family of membrane proteins specialized in rapid water conduction across biological membranes. Whether these channels also conduct gas molecules and the physiological significance of this potential function have not been well understood. Here we report 140 ns of molecular dynamics simulations of membrane-embedded AQP1 and of a pure POPE bilayer addressing these questions. The permeability of AQP1 to two types of gas molecules, O₂ and CO₂, was investigated using two complementary methods, namely, explicit gas diffusion simulation and implicit ligand sampling. The simulations show that the central (tetrameric) pore of AQP1 can be readily used by either gas molecule to permeate the channel. The two approaches produced similar free energy profiles associated with gas permeation through the central pore: a −0.4 to −1.7 kcal/mol energy well in the middle, and a 3.6–4.6 kcal/mol energy barrier in the periplasmic vestibule. The barrier appears to be mainly due to a dense cluster of water molecules anchored in the periplasmic mouth of the central pore by four aspartate residues. Water pores show a very low permeability to O₂, but may contribute to the overall permeation of CO₂ due to its more hydrophilic nature. Although the central pore of AQP1 is found to be gas permeable, the pure POPE bilayer provides a much larger cross-sectional area, thus exhibiting a much lower free energy barrier for CO₂ and O₂ permeation. As such, gas conduction through AQP1 may only be physiologically relevant either in membranes of low gas permeability, or in cells where a major fraction of the cellular membrane is occupied by AQPs.

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Keywords: Aquaporin; AQP1; Gas permeability; O₂; CO₂; Free energy profile; Gas channels

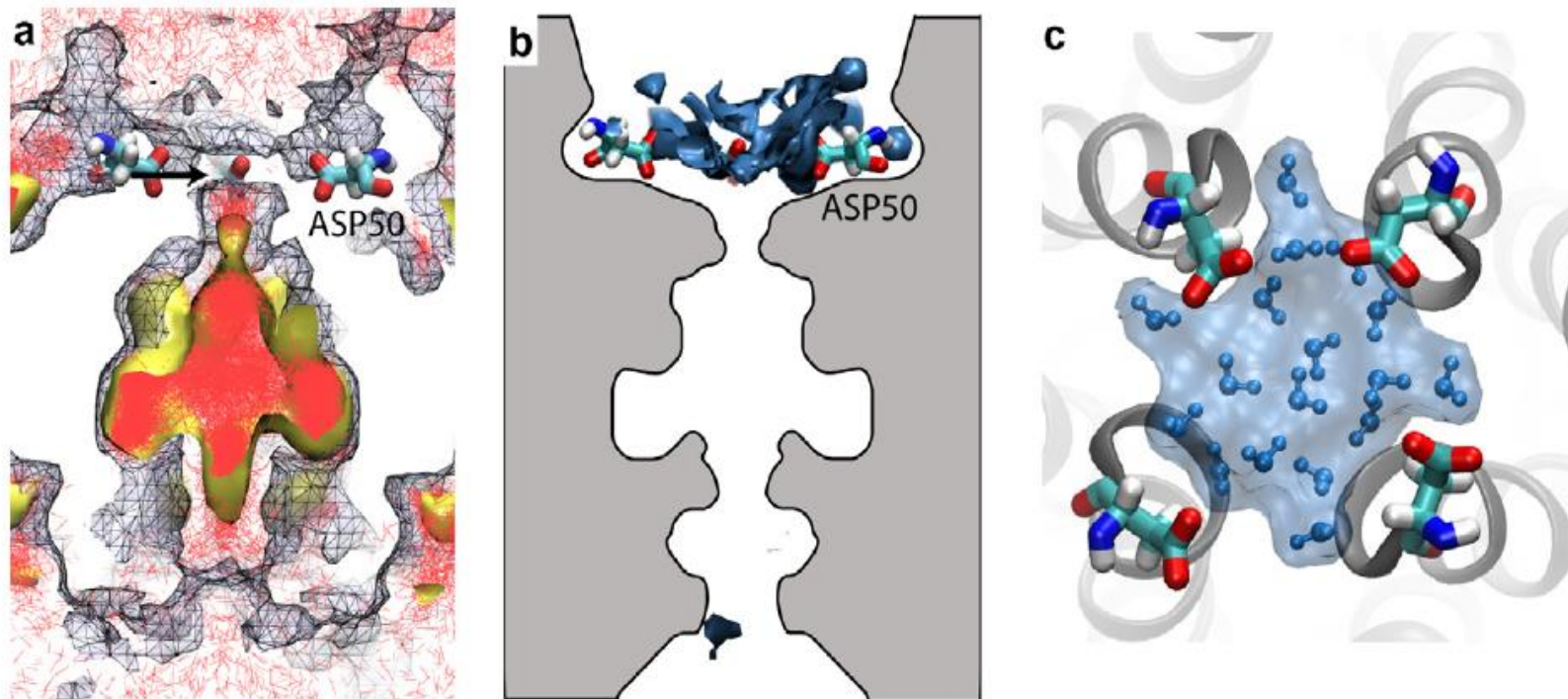


Fig. 6. (a) Close-up of the energy barrier (indicated by the black arrow) at the periplasmic mouth of the AQP1 central pore. The 0 kcal/mol (yellow) and 3 kcal/mol (black) energy isosurfaces calculated from implicit ligand sampling are shown, along with O₂ positions obtained from explicit gas diffusion simulations (red). (b) Water occupancy per Å³, showing in blue the volumes occupied by water during at least 75% of the time during the 26-ns AQP1-O₂ simulation. A layer of water, denser than the bulk, is found around the Asp50 residues, corresponding to the location of the energy barrier shown in (a). Water occupancy was calculated using the volmap plugin of VMD (Humphrey et al., 1996). The profile of the central pore is shown in black lines. (c) Snapshot of water molecules around Asp50 during equilibrium simulations of AQP1-O₂. The water molecules are shown in both surface and CPK representations.

According to our PMFs, the major barrier of the central pore to gas permeation is about 3.6–4.6 kcal/mol, located at the periplasmic side of the central pore ($13 \text{ Å} \leq z \leq 19 \text{ Å}$). This barrier, consistently found by both our approaches, as well as by another study (Hub and de Groot, 2006), surprisingly, does not correspond to a region that is sterically blocked directly by the protein. As shown in Fig. 6a, this barrier is located above the region of maximum protein contraction where the four hydrophobic residues Val52 reside; rather, it corresponds to a region that is populated solely by water. We have created a volumetric map of the local occupancy of water, as shown in

Fig. 6b. It is clear that the barrier corresponds to a dense layer of water molecules surrounded by four aspartate (Asp50) residues (Fig. 6c). With a higher density than the bulk water, this water layer reduces the chance of gas molecules to access the central pore. If these aspartate residues will be mutated to neutral residues, e.g., alanines or asparagines, the strong electrostatic effects of the quadruplets may be eliminated and a less dense water structure could be expected, which might result in a better gas-conductive central pore of AQP1. Simulations of these mutants are currently underway.

Protein sequence alignment

D50 **V52**

bAQP1.PRO MASEFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQDNVKVSLAFGL 60
hAQP5.PRO MKKEVCSPAFLKAVFAEFLATLIFVFFGLGSALKWPS-----ALPTILQIALAFGL 51
* . * . . * : ** . ***** ::*: *: :.***** :

T41 L43

bAQP1.PRO SIATLAQSVDGHISGAHLNPAVTLGILLSCQISVLRAIMYIIAQCVGAIVATAILSGITSS 120
hAQP5.PRO AIGTLAQALGPVSGGHINPAITLALLVGNQISLLRAFFYVAAQLVGAIAGAGILYGVAPL 111
:* . *****::* : ** . *:***: ** . ** : . ***:***: **: ** ***** ..: ** *::.

bAQP1.PRO LPDNSLGLNALAPGVNSGQGLGIEIIGTLQLVLCVLATTDRRRDLGGSGPLAIGFSVAL 180
hAQP5.PRO NARGNLAVNALNNNTTQGQAMVVLEILT FQLALCIFASTDSRRTSPVGPALSIGLSVTL 171
. . . * .:*****.: :*: * *:*.**:*:** ** . ** .*:**:*:*:

bAQP1.PRO GHLLAIDYTGCGINPARSFGSSVITHNFQ-DHWIFWVGPFIGAALAVLIYDFILAPRSSD 239
hAQP5.PRO GHLVGIYFTGCSMNPARSFPAVVMNRFS PAHWWFVGPIVGAVLAAILYFYLLFPNSLS 231
:.* :. :*****. :*: :.* **:****:***.**.:* :*: *. *

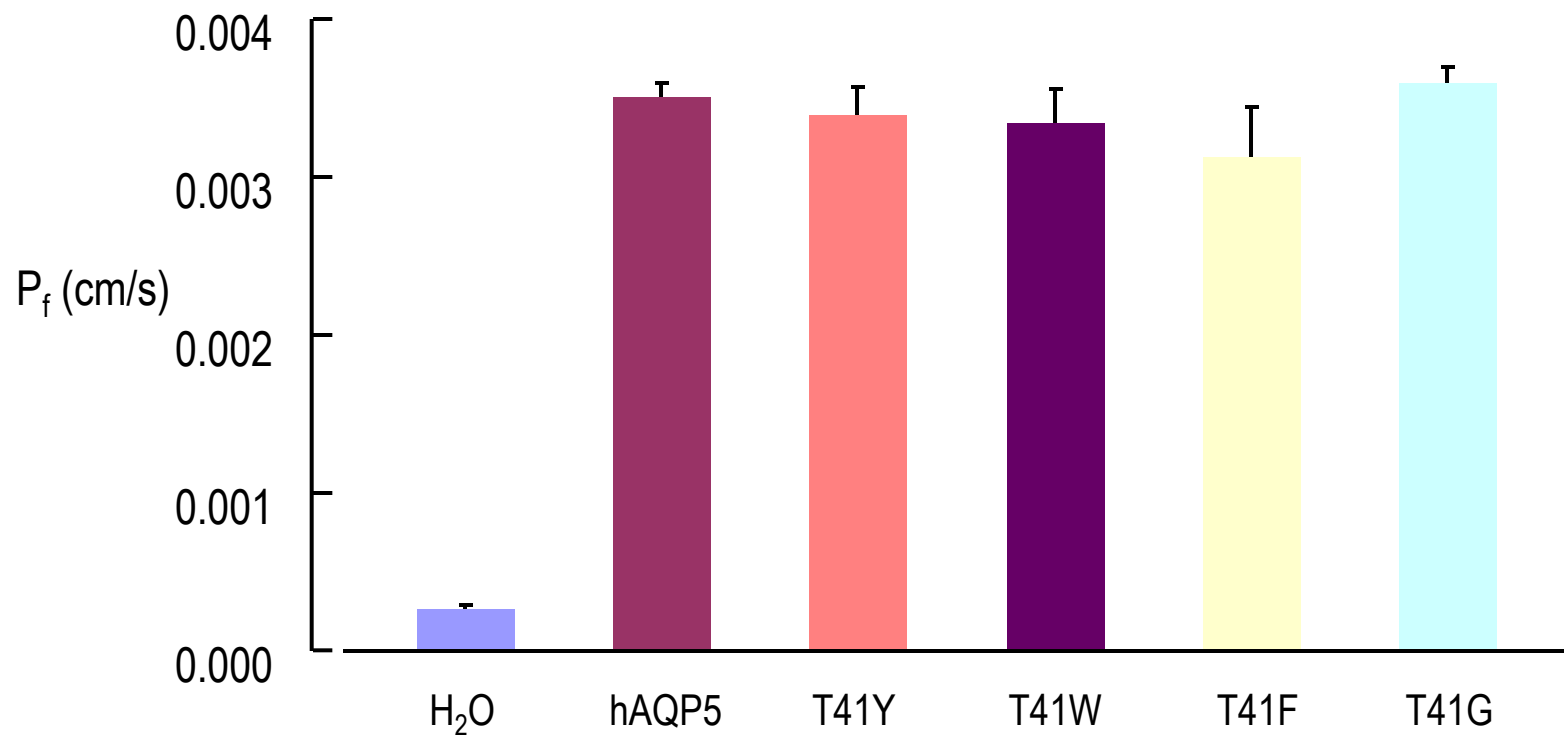
bAQP1.PRO LTDRVKVWTSG--QVEEYDL DADDINSRVEMKPK 271
hAQP5.PRO LSERVAIIKGTYEPDEDWEEQREERKKTMELTTR 265
*:*** :.. *:::: : : : :*:::

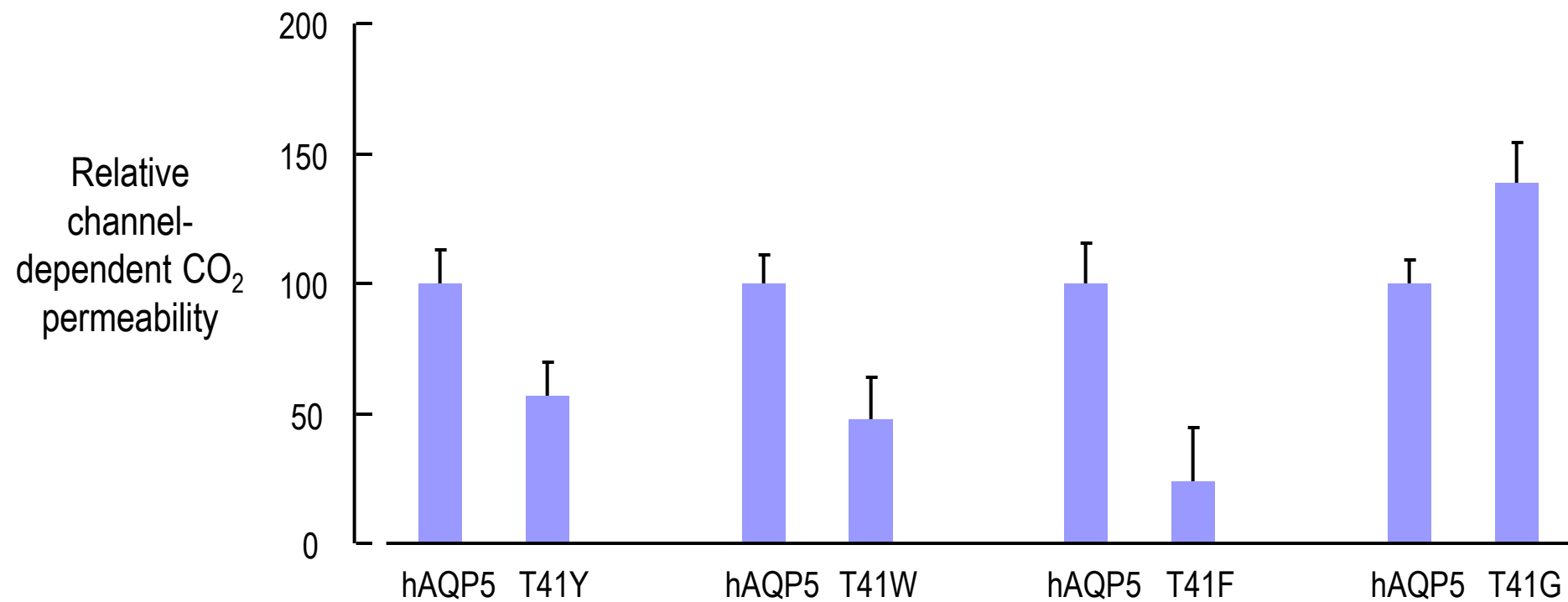


bAQP1



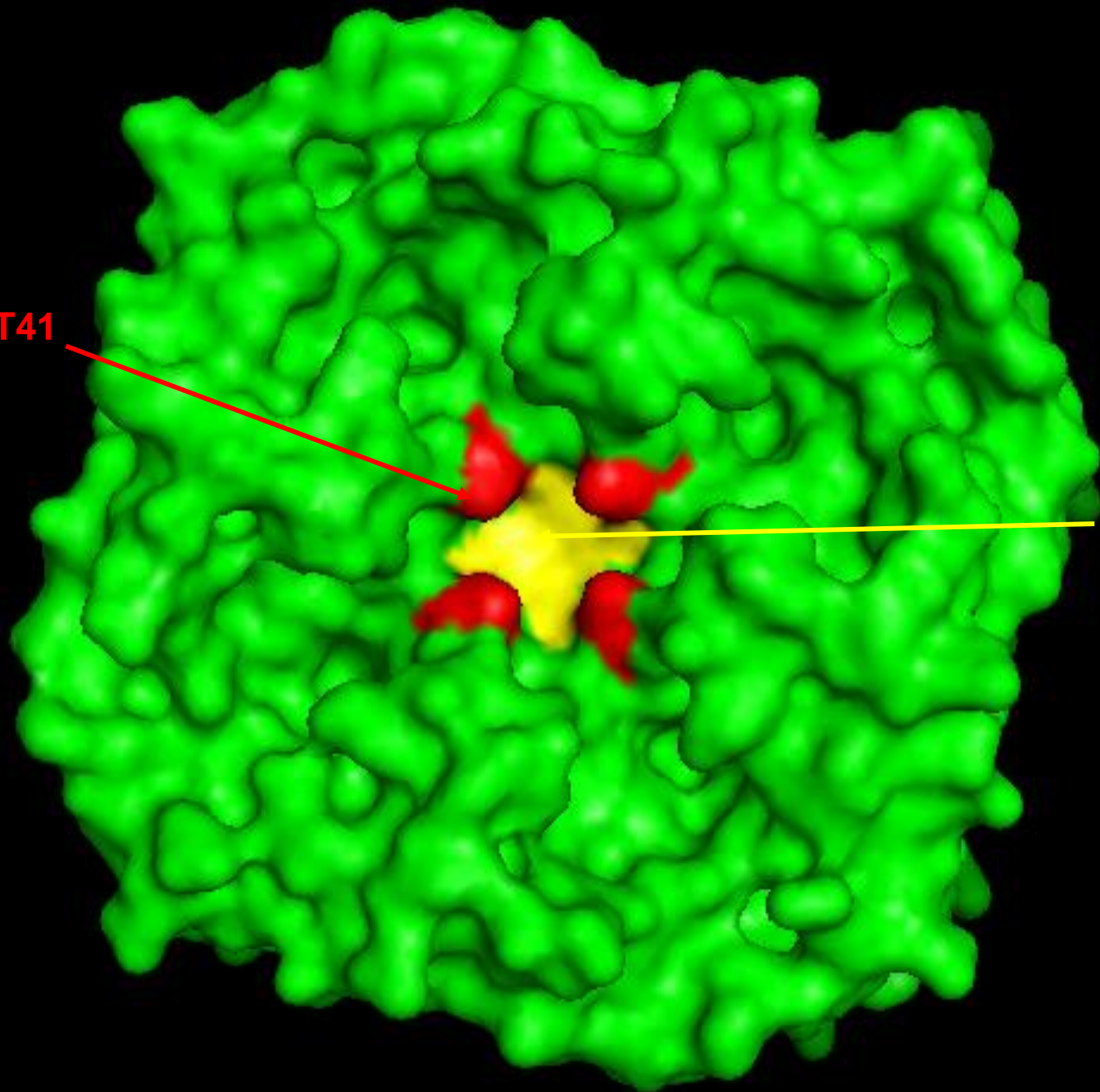
hAQP5

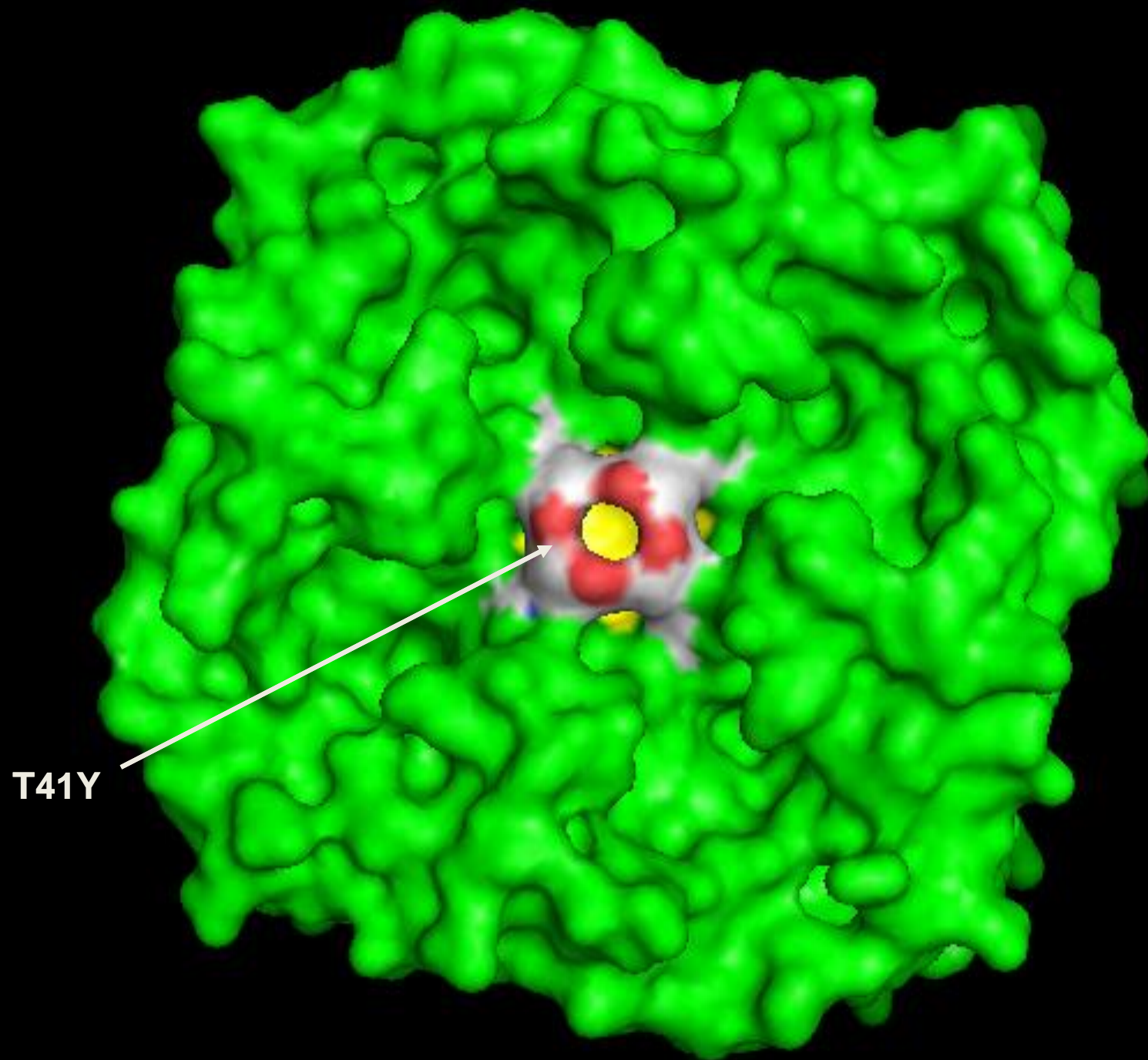


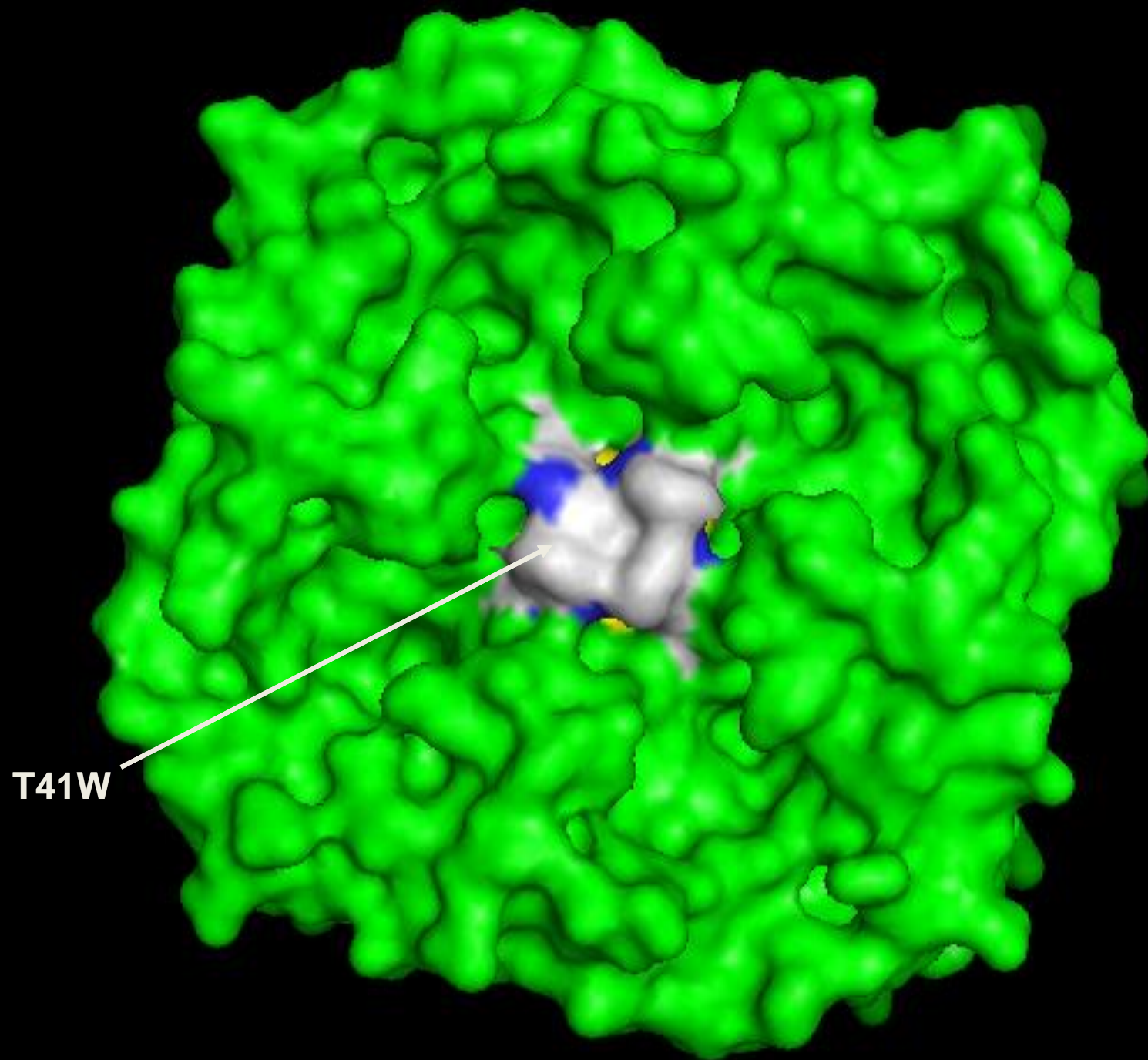


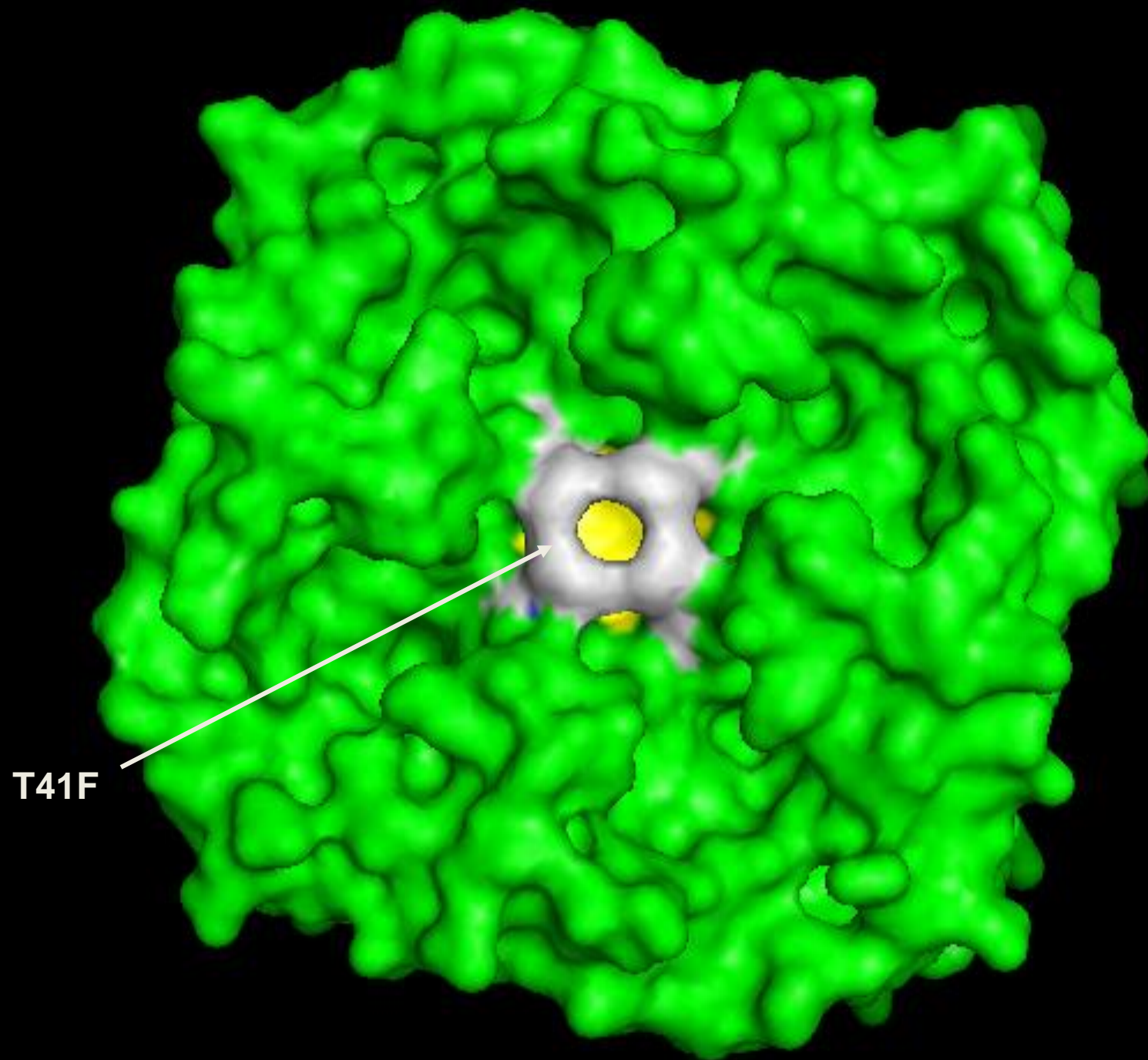
T41

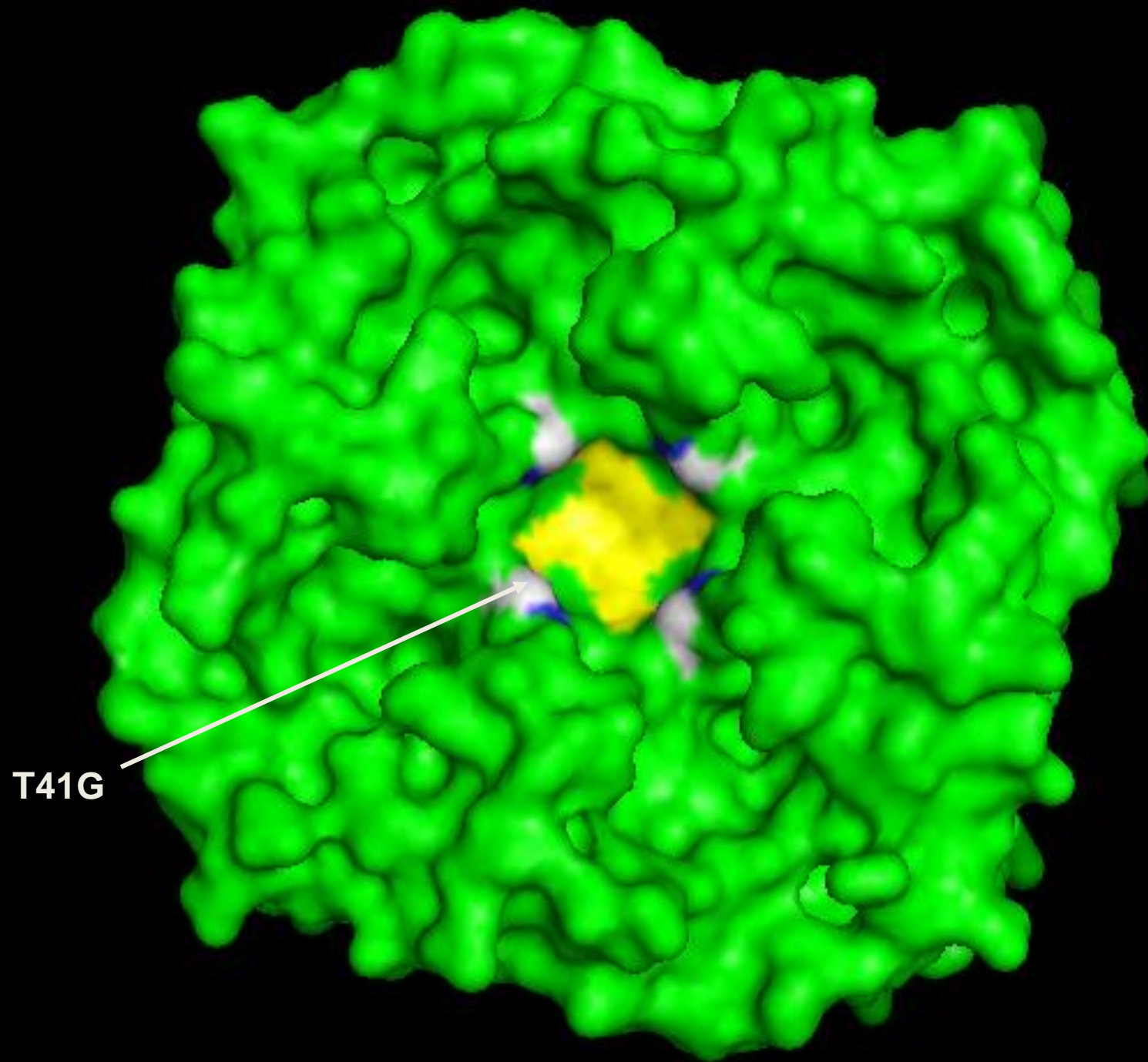
L43











Conclusion I

I Amino acids at the mouth of the central pore

Changes of $\Delta p H_s$ (CO_2 permeability) is more sensitive than P_f (H_2O permeability)

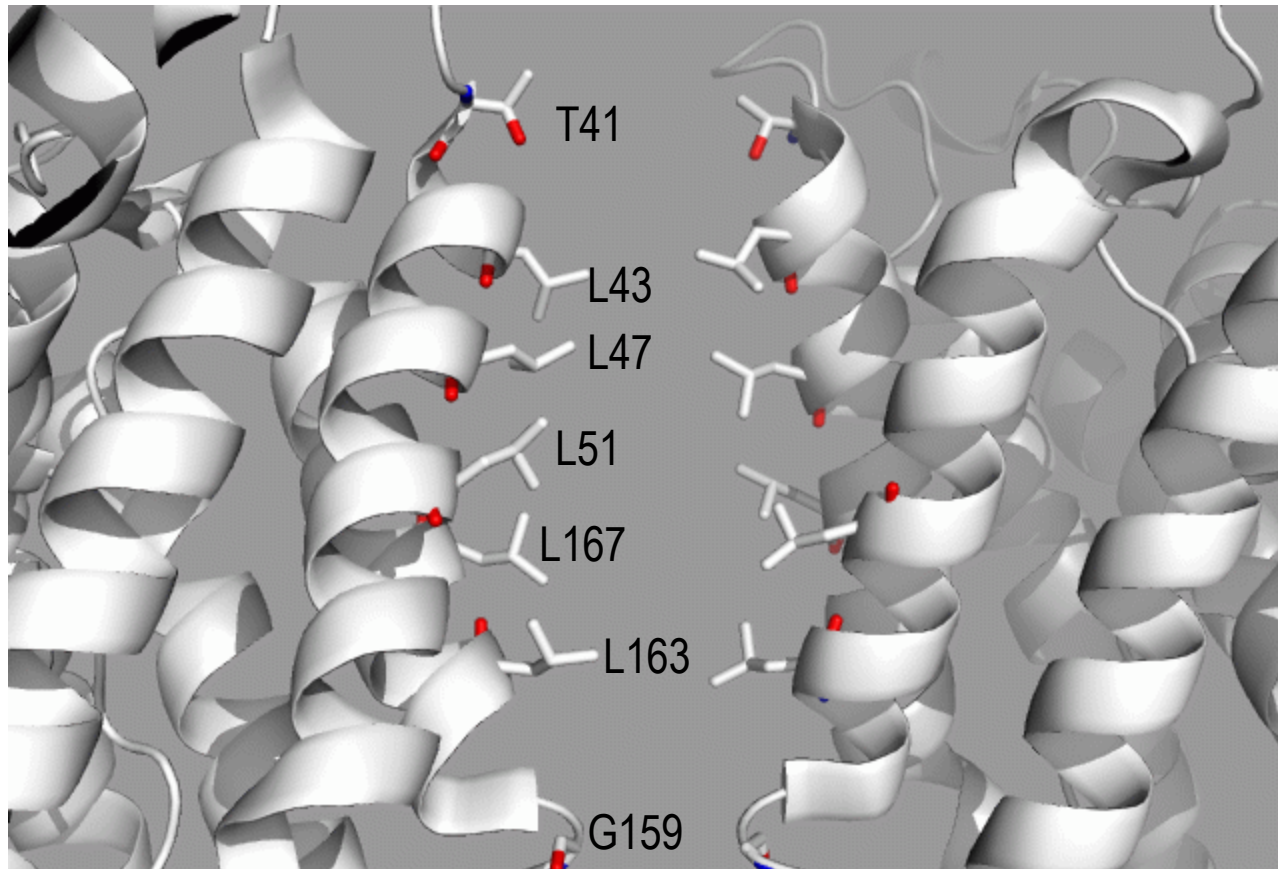
T41 is more important than L43.

II Amino acids lining the central pore

Outline

- I Amino acids at the mouth of the central pore
- II Amino acids lining the central pore

Amino acids lining the central pore



Sequence alignment of AQPs

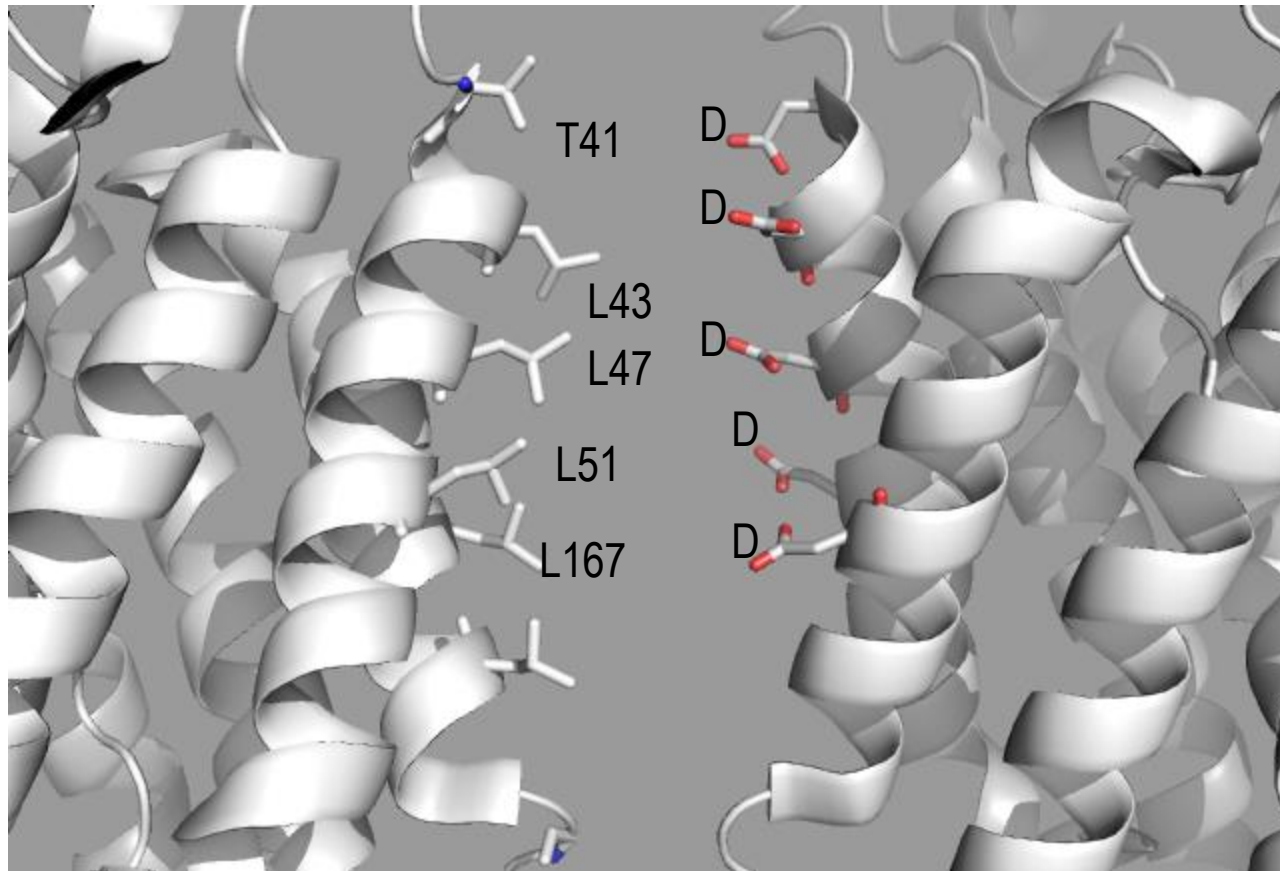
hAQP9.PRO	GCGCVAQAILS	-----	GRFGGV	ITIN	VGF	SM	AVAMAIYVAGGV	SGGHINPAVSLAMCL	93		
hAQP10.PRO	TQGA	VAQAVTSG	-----	ETKGNFF	TMFL	AGSL	AVTIAIYVGGNV	SGAHLNPAFSLAMCI	91		
hAQP3.PRO	GCGS	VAQVVLSR	-----	GTHGGFL	TINL	AFGF	FAVTLGILIAGQV	SGAHLNPAVTFAMCF	92		
hAQP7.PRO	GLGS	VAHMLN	-----	KKYGSY	LGVN	IGF	FGVTMGVHVAG	RISGAHMNAAVTFANCA	103		
hAQP2.PRO	GLGS	ALNWPQ	-----	ALPSVL	QIAMA	FGLG	IGTLVQALGHIS	GAHINPAVTVACL	77		
hAQP5.PRO	GLGS	ALKWPS	-----	ALPT	ILQIAL	AFGL	LAIGTLAALGPV	SGGHINPAITLALLV	78		
hAQP6.PRO	GVGS	VMRWPT	-----	ALPSVL	QIAIT	FNLT	VTAMAVQVTWK	ASGAHANPAVTLAFLV	91		
bAQP0.PRO	GLGAS	LRWAP	-----	GPLHVL	QVAL	AFGL	ALATLVQAVGHIS	GAHVNPVAVTFAFLV	77		
hAQP4-M1.PRO	SLGST	INWG	---	GTEKP	--	LPVDMVLIS	LCFGLSIATMVQCF	GHISGGHINPAVTVAMVC	106		
hAQP4-M23.PRO	SLGST	INWG	---	GTEKP	--	LPVDMVLIS	LCFGLSIATMVQCF	GHISGGHINPAVTVAMVC	84		
bAQP1.PRO	SIGS	ALGFHYPIK	SNQTTGAVQDN	VKVS	LA	FGLS	IAATLAQSVGHIS	GAHLNPAVTLGLLL	87		
hAQP1.PRO	SIGS	ALGF	KYPVGNNQT	--	AVQDN	VKVS	LA	FGLS	IAATLAQSVGHIS	GAHLNPAVTLGLLL	85
hAQP8.PRO	GCLSV	VIENG	-----	TD	TGLLQ	PALAHGL	LALGLVIATLGNI	SGGHFNPAVSLAAML	101		
hAQP11.PRO	LCCCTH	ELQLLS	-----	EQHP	AHPTW	TLTL	LVYFFSLVHGLT	LVGTSSNPCGVMMQMM	108		

TM2

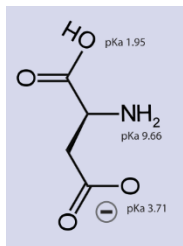
hAQP9.PRO	YPAPYLSLANAFADQ	VVATMILLIIVFAIFDS	RNLGAP	R	GLEPT	IAIGLLIIVIAS	SLGLN	210
hAQP10.PRO	YPAPYLSLNNGFLD	QVLGTGMLIVGLLAILDR	R	NKGV	PAGLEP	VVGM	LILALGLSMGAN	208
hAQP3.PRO	YPSGHLDMINGFFD	QFIGTASLIVCVLAIVDPYNN	PVPR	R	GLEAFT	VGLVVLVIGT	SMGFN	209
hAQP7.PRO	YLPDHMTLWRGFL	NEAWLTGMLQLCLFAITDQ	ENNPALP	GTEAL	VIGILVVIIGV	SLGMN	220	
hAQP2.PRO	ALSNSTTAGQAVT	VELFLTLQLVLCIFASTD	ERRGENP	GTPALS	SIGFS	SVALGHLLGIHY	178	
hAQP5.PRO	ALNNNTTQGQAMV	VELILTFQLALCIFASTD	SRRTSPV	GSPALS	SIGLSVT	LGHLVGIYF	179	
hAQP6.PRO	VVRNSVSTGQAV	AVELLTLQLVLCVFASTD	SRQTS	--	GSPATMIGIS	SVALGHLIGIHF	190	
bAQP0.PRO	TLHPGVS	VGQATIVEIFLTLQFVLCIFATYD	ERRNGRL	GSVAL	AVGFS	LT	LGHLFGMY	178
hAQP4-M1.PRO	MVHGNLTAGHGLL	VELIITFQLVFTIFASCD	SKRTDVT	GSIALA	IGFS	VAIGHLFAINY	207	
hAQP4-M23.PRO	MVHGNLTAGHGLL	VELIITFQLVFTIFASCD	SKRTDVT	GSIALA	IGFS	VAIGHLFAINY	185	
bAQP1.PRO	ALAPGVNSGQGLG	IEIIGTLQLVLCVLATTD	RRRRDLG	SGPLA	IGFS	SVALGHLLAIDY	188	
hAQP1.PRO	DLADGVNSGQGLG	IEIIGTLQLVLCVLATTD	RRRRDLG	GSAPLA	IGLS	SVALGHLLAIDY	186	
hAQP8.PRO	TVQEQGQVAGAL	VAEIIITLLALAVCMGAIN	EKT	KGP	--	LAPFSIGFAVTVD	ILAGGPV	204
hAQP11.PRO	RSFACKNPIRV	DLKAVITEAVCSFLFHSALLHFQ	EVRT	K	LR	IHLAALITFLVYAGGSL	210	

TM5

If mutate to Asp (D)

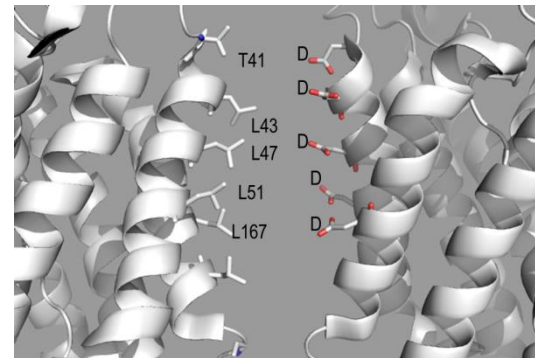
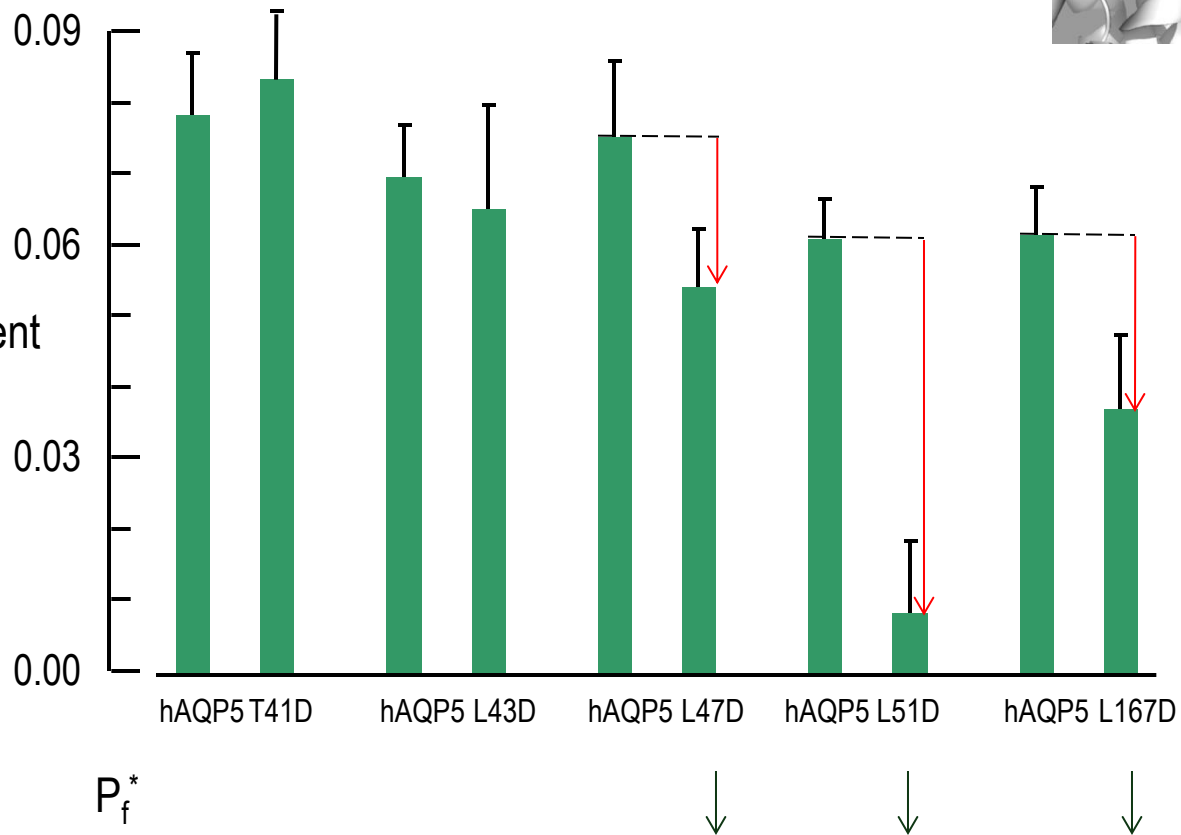


Aspartic Acid (Asp)

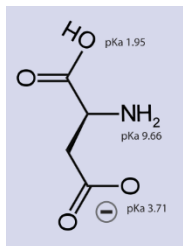


LXXD - ΔpH_s^*

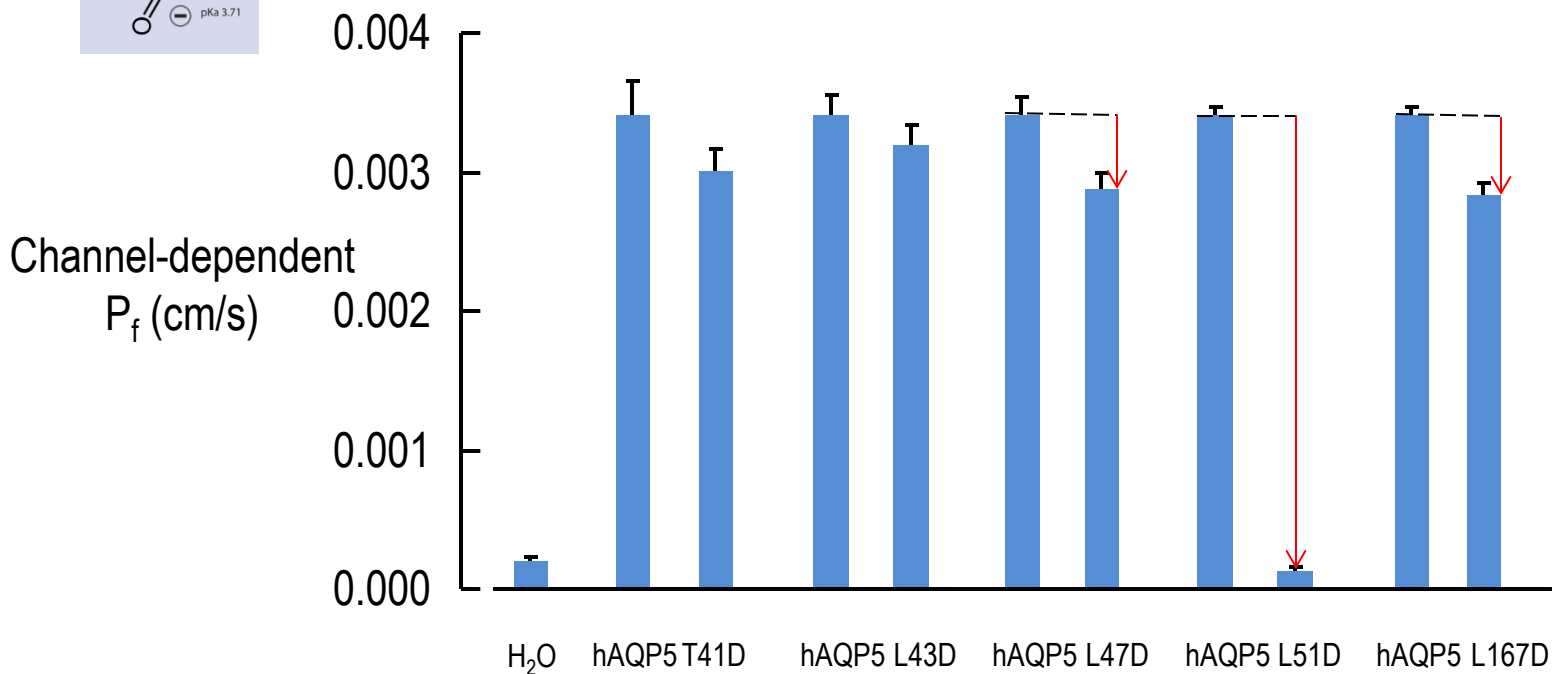
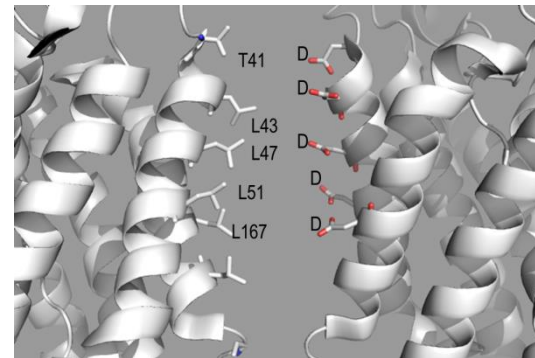
Channel-dependent
 ΔpH_s for CO₂



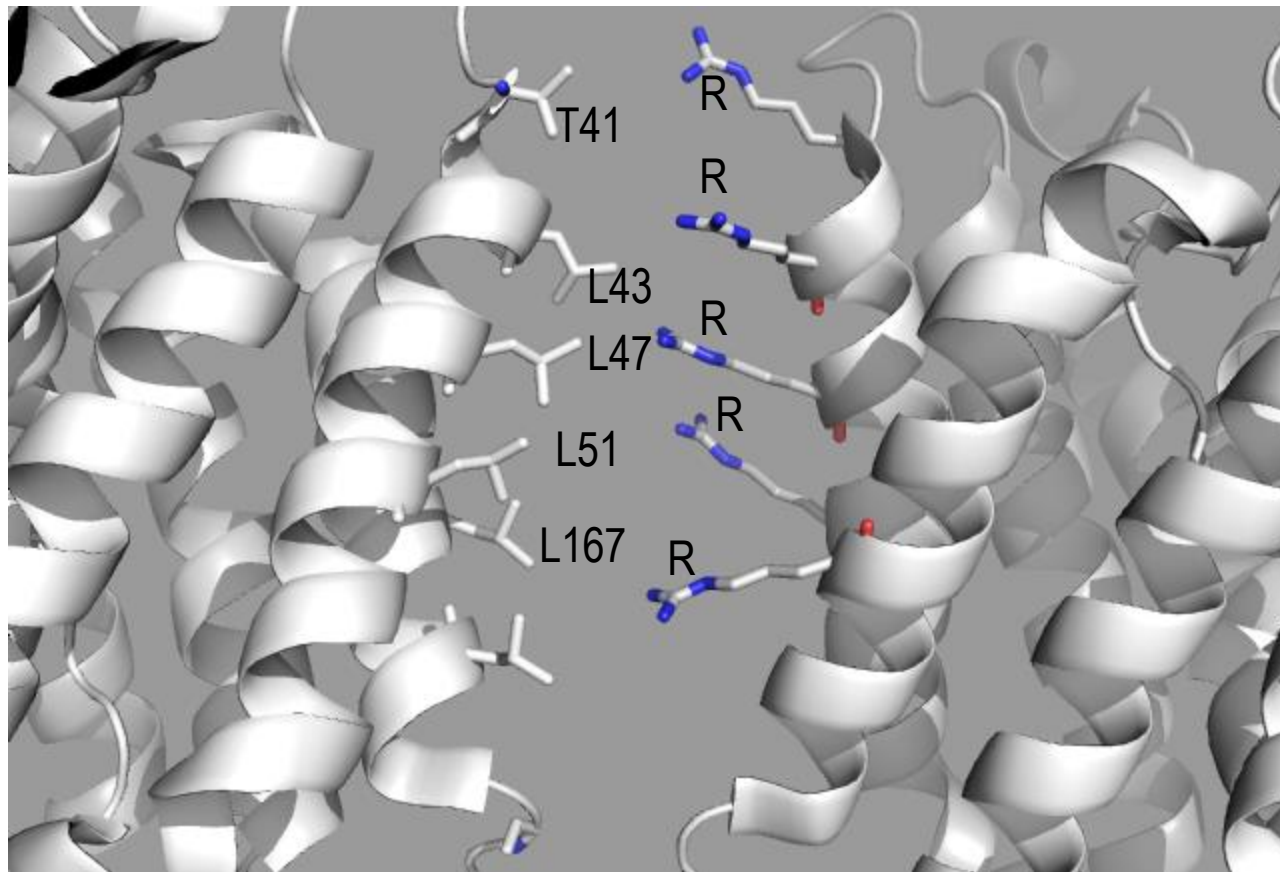
Aspartic Acid (Asp)



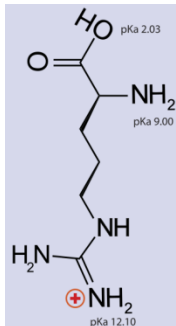
LXXD - P_f^{*}



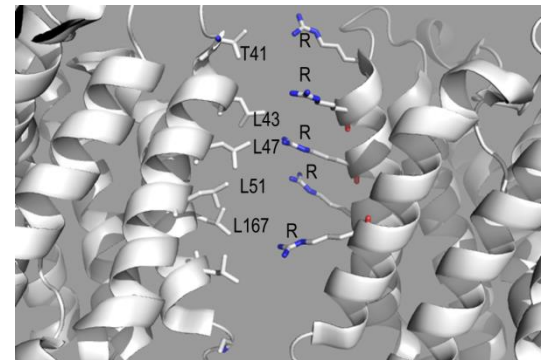
If mutate to Arg (R)



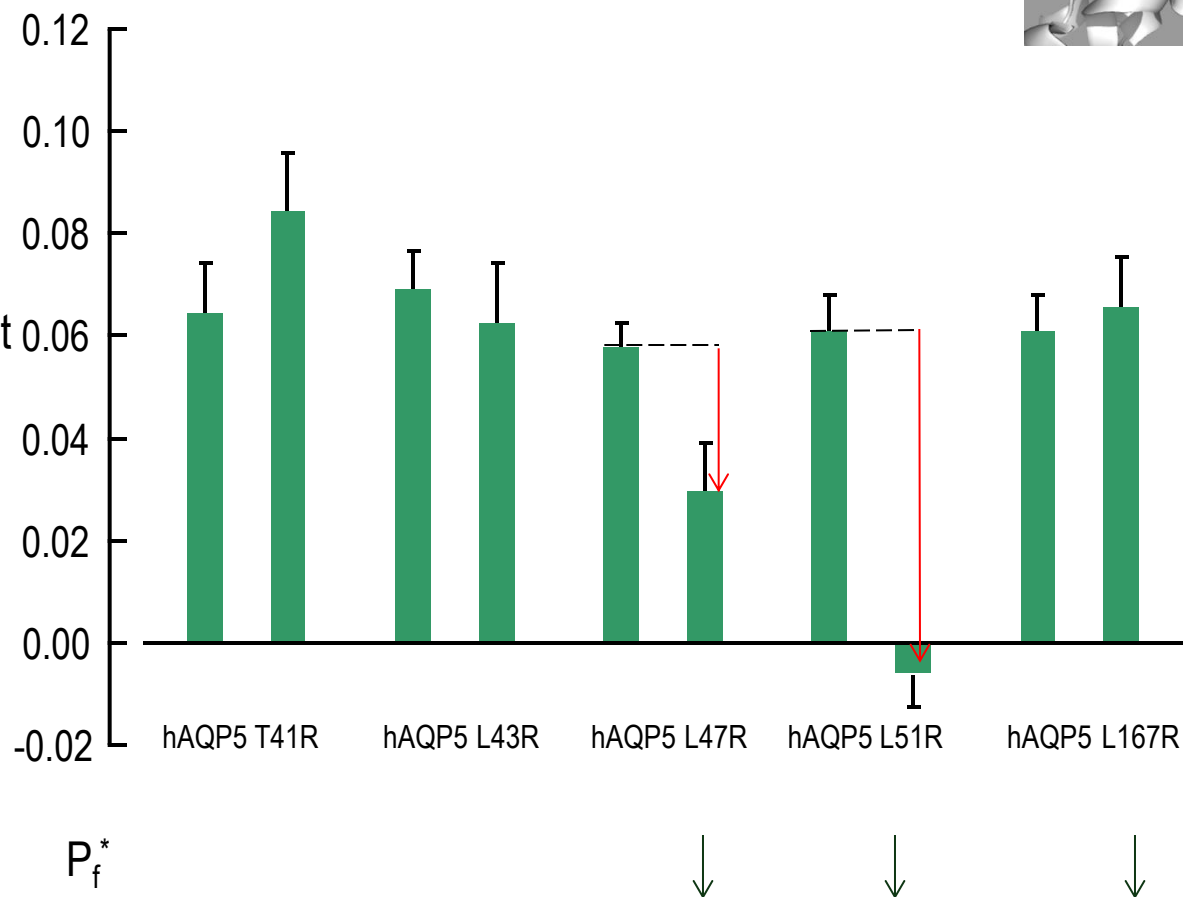
Arginine (Arg)



LXXR - ΔpH_s^*



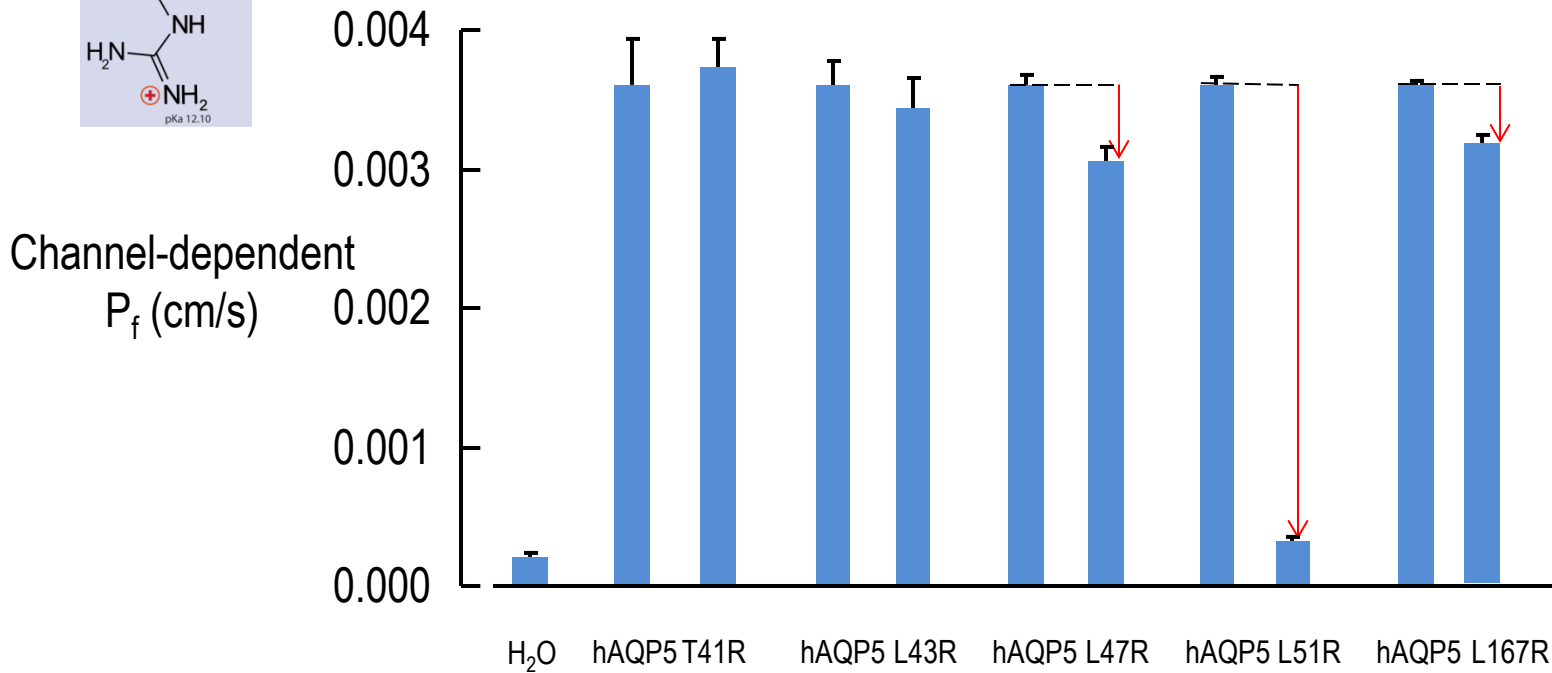
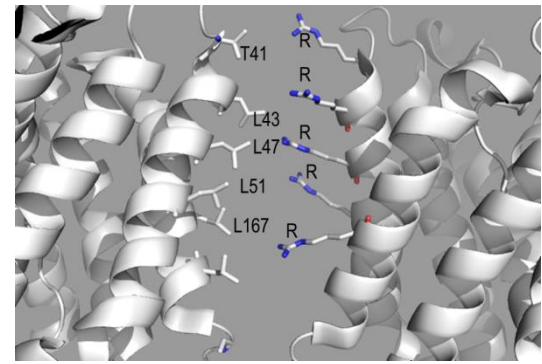
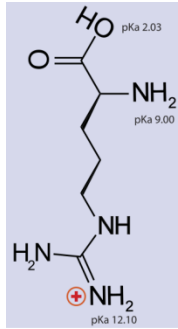
Channel-dependent
 ΔpH_s for CO_2



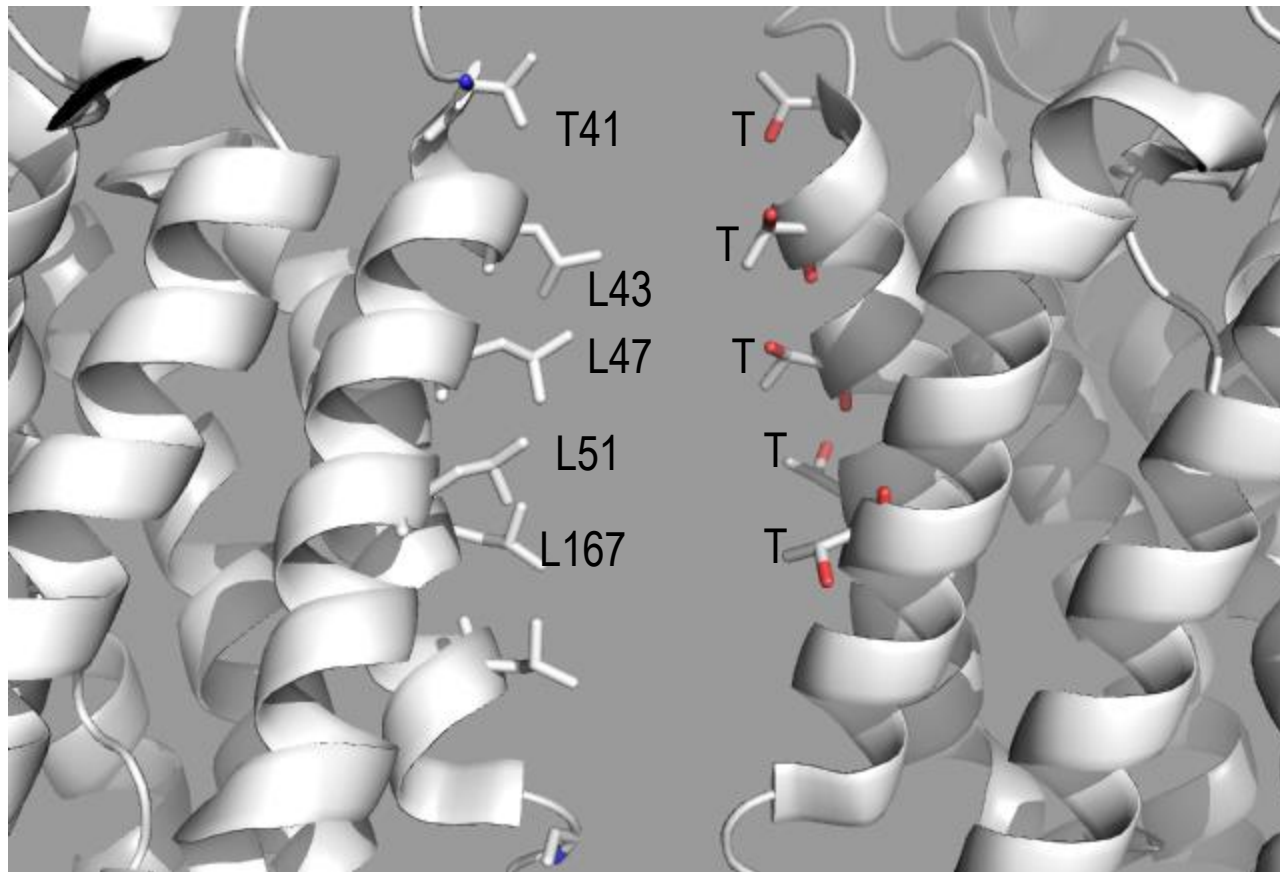
P_f^*

LXXR - P_f^{*}

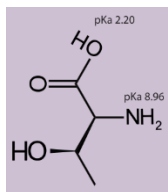
Arginine (Arg)



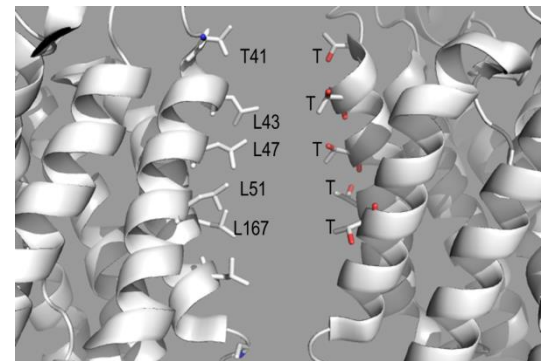
If mutate to Thr (T)



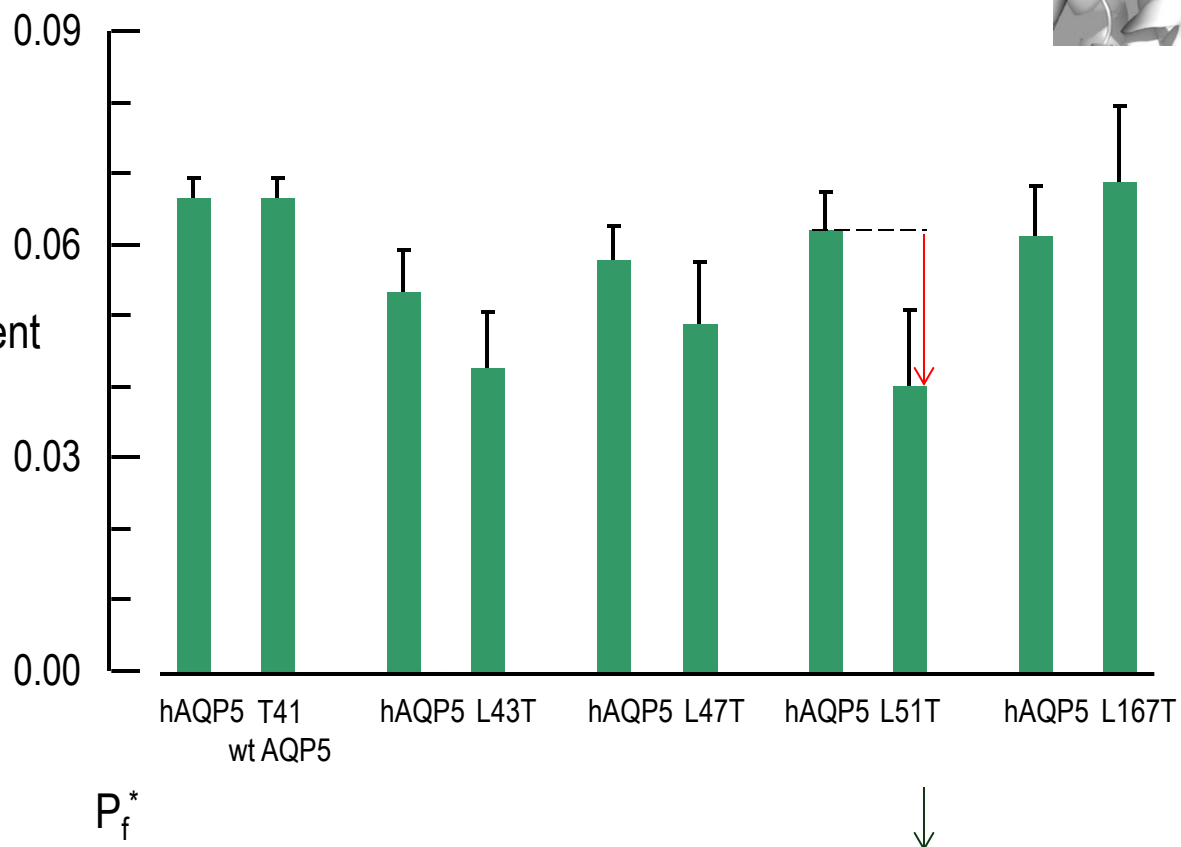
Threonine (Thr)



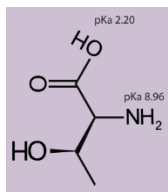
LXXT - ΔpH_s^*



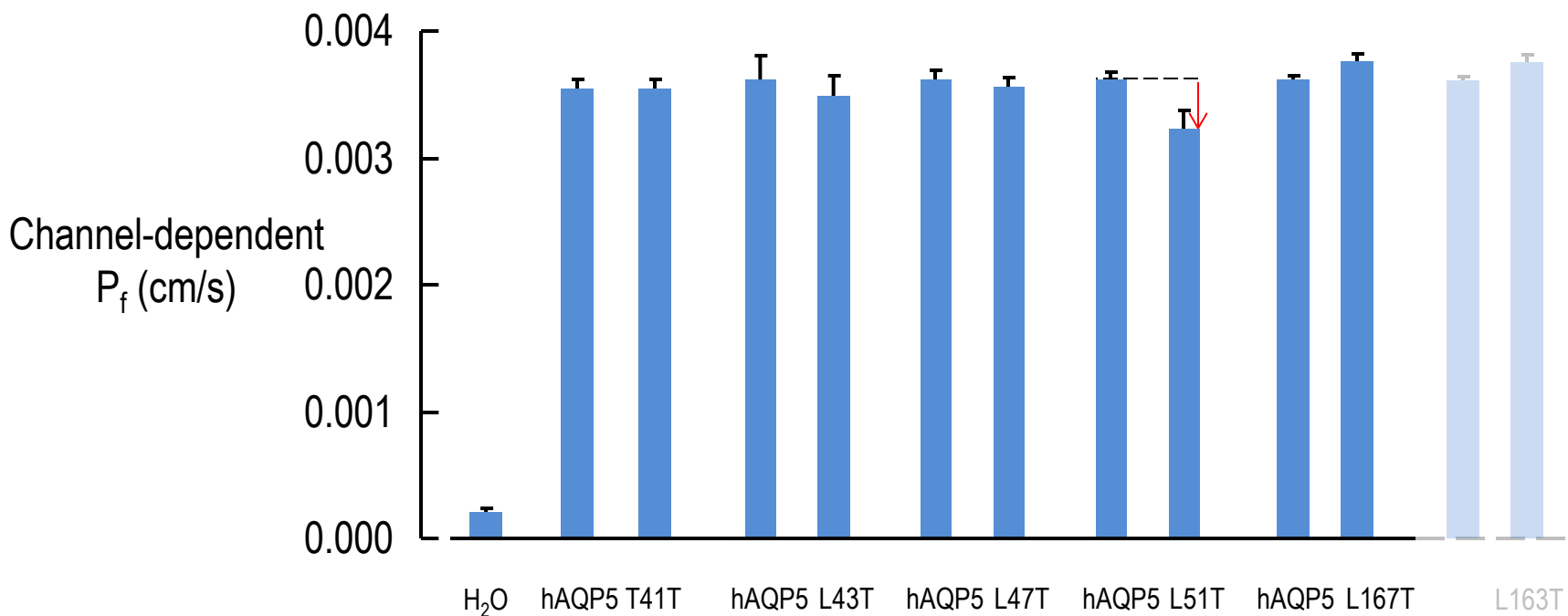
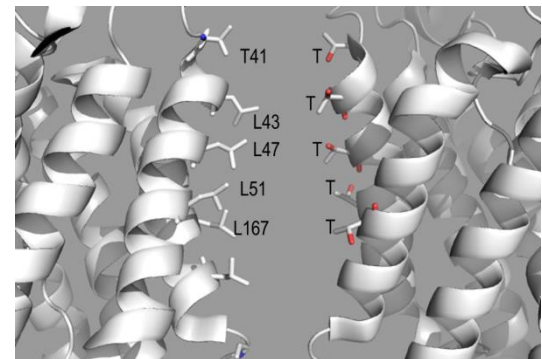
Channel-dependent
 ΔpH_s for CO₂



Threonine (Thr)



LXXT - P_f^*



Conclusion II

I Amino acids at the mouth of the central pore

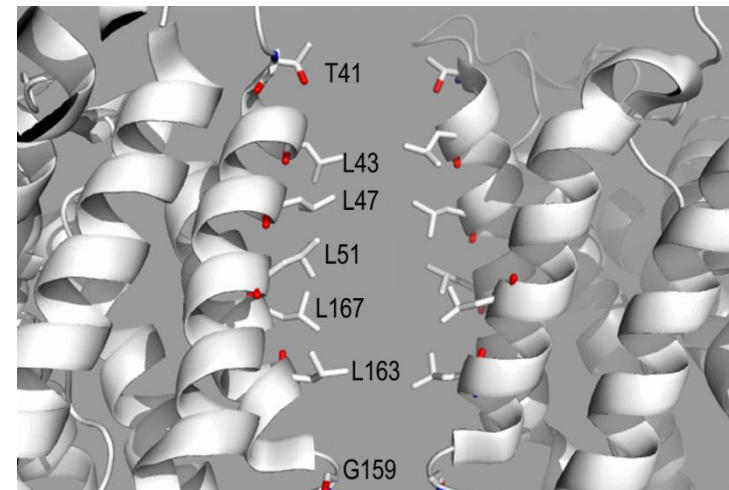
Changes of ΔpH_s (CO_2 permeability) is more sensitive than P_f (H_2O permeability)

T41 is more important than L43.

II Amino acids lining the central pore

Changes of P_f (H_2O permeability) is more sensitive than ΔpH_s (CO_2 permeability)

Of all the amino acids lining the central pore, L51 is most sensitive to determine P_f and ΔpH_s .



Acknowledgement

Collaborator

Emad Tajkhorshid

Lab

Walter F Boron

Raif Musa-Aziz

Mark D Parker



**American
Heart
Association®**
Learn and Live

Gas Channels Workshop

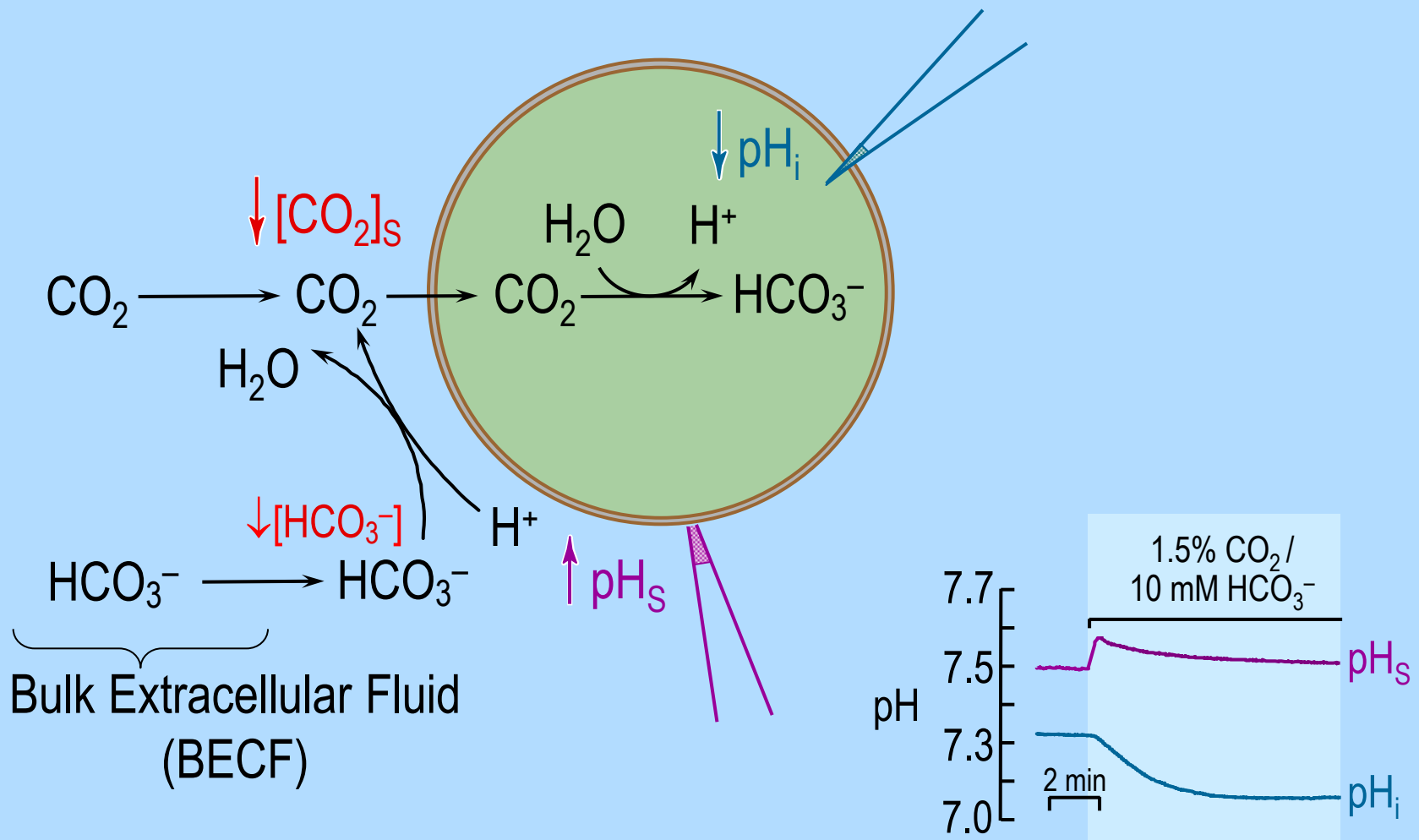
***September 7, 2012
Cleveland, Ohio***

Mathematical Modeling of Gas Movements in an Oocyte

Rossana Occhipinti, Ph.D.

Department of Physiology & Biophysics
Case Western Reserve University School of Medicine
10900 Euclid Avenue
Cleveland, OH 44106-4906

Xenopus oocyte: pH Changes Caused by CO₂ Influx

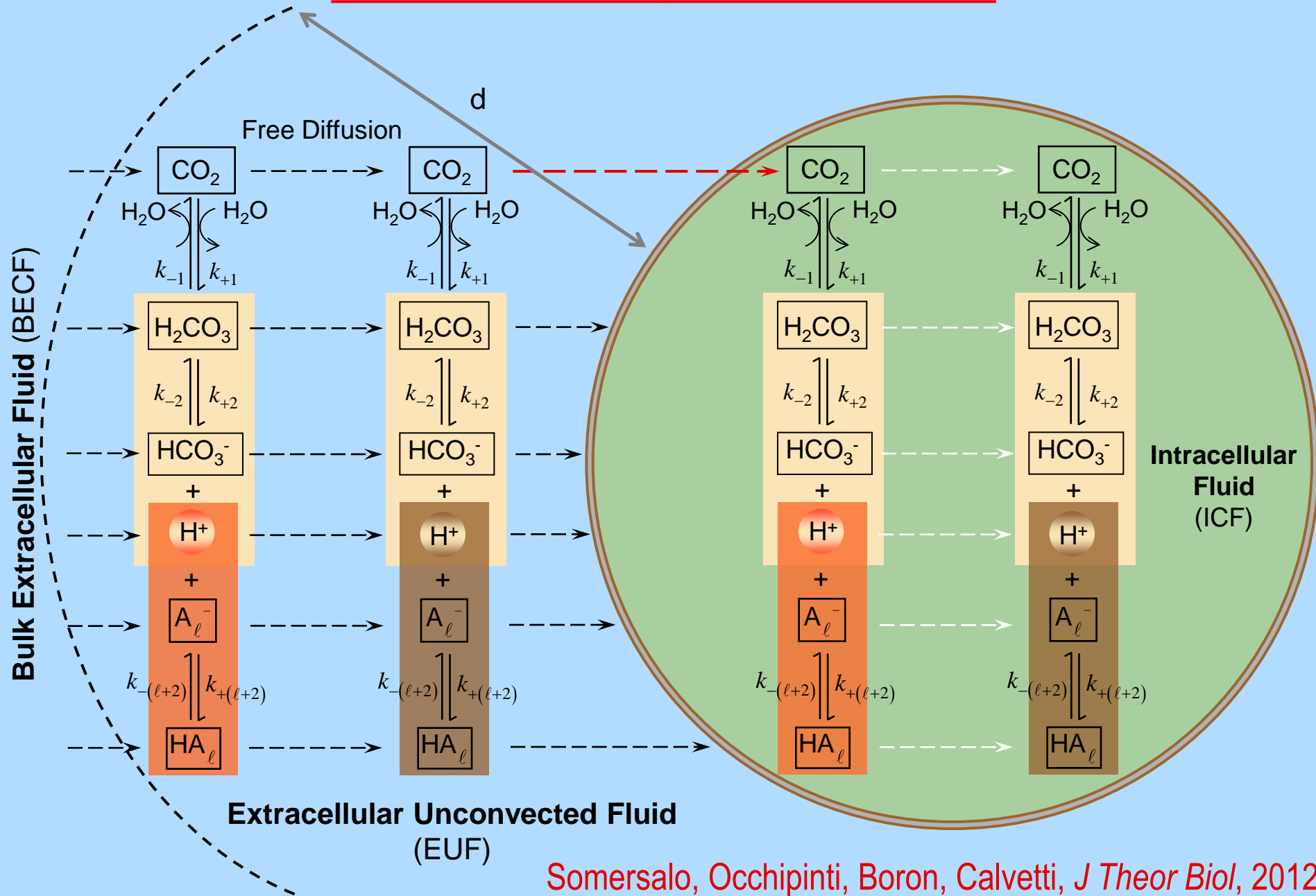


(data kindly provided by Dr. Musa-Aziz)

An appropriate mathematical model should include...

- A spherical cell
- Transport of CO_2 across the plasma membrane
- Reactions of a multitude of extra- and intracellular buffers
- Diffusion of solutes through the extra- and intracellular spaces
- Temporal and spatial variations of solute concentrations
- Carbonic anhydrase (CA) activity at specific loci

The Mathematical Model



The Key Components of the Model

Bulk extracellular fluid (BECF)

Infinite reservoir where convection could occur but not reaction or diffusion

Extracellular unconvected fluid (EUF)

Thin layer adjacent to the surface of the oocyte where no convection occurs, but reactions and diffusion do occur

Plasma membrane

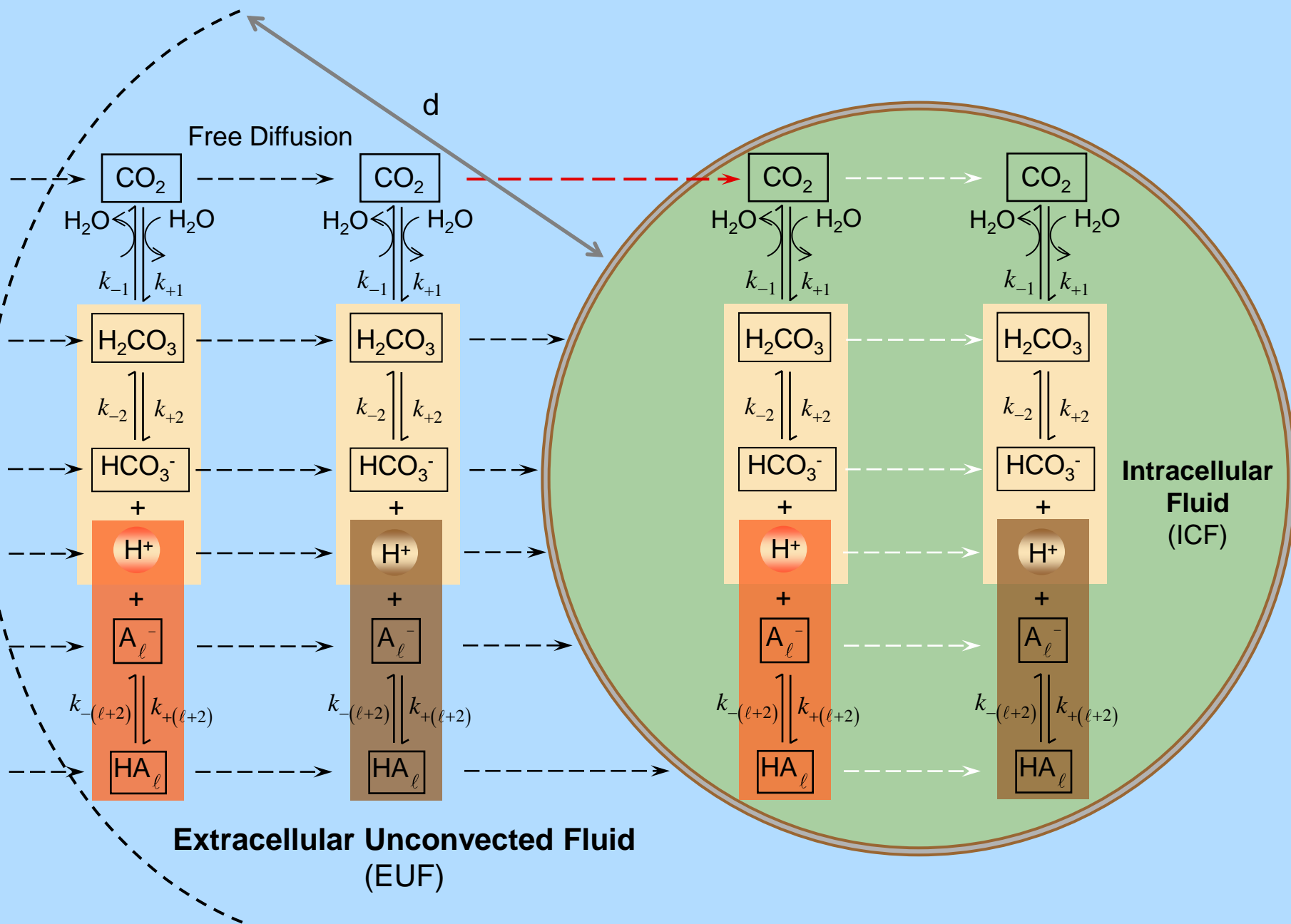
Infinitely thin and permeable only to CO_2

In both EUF and intracellular fluid (ICF)

Slow equilibration of the CO_2 hydration/dehydration reactions

Competing equilibria among the $\text{CO}_2/\text{HCO}_3^-$ and a multitude of non- $\text{CO}_2/\text{HCO}_3^-$ buffers

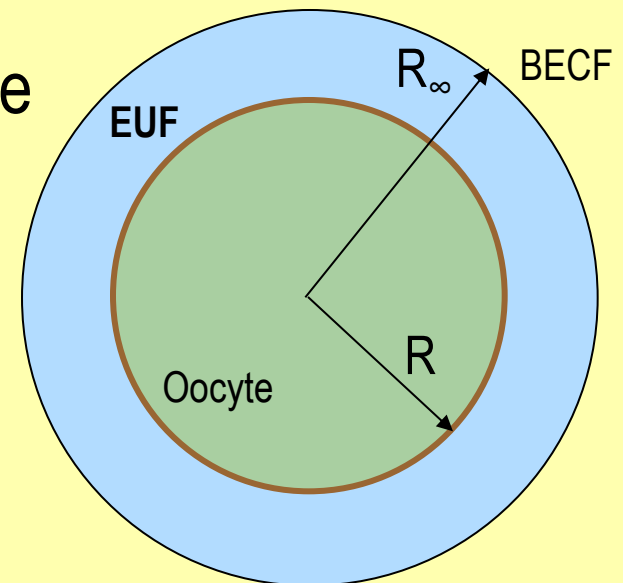
Bulk Extracellular Fluid (BECF)



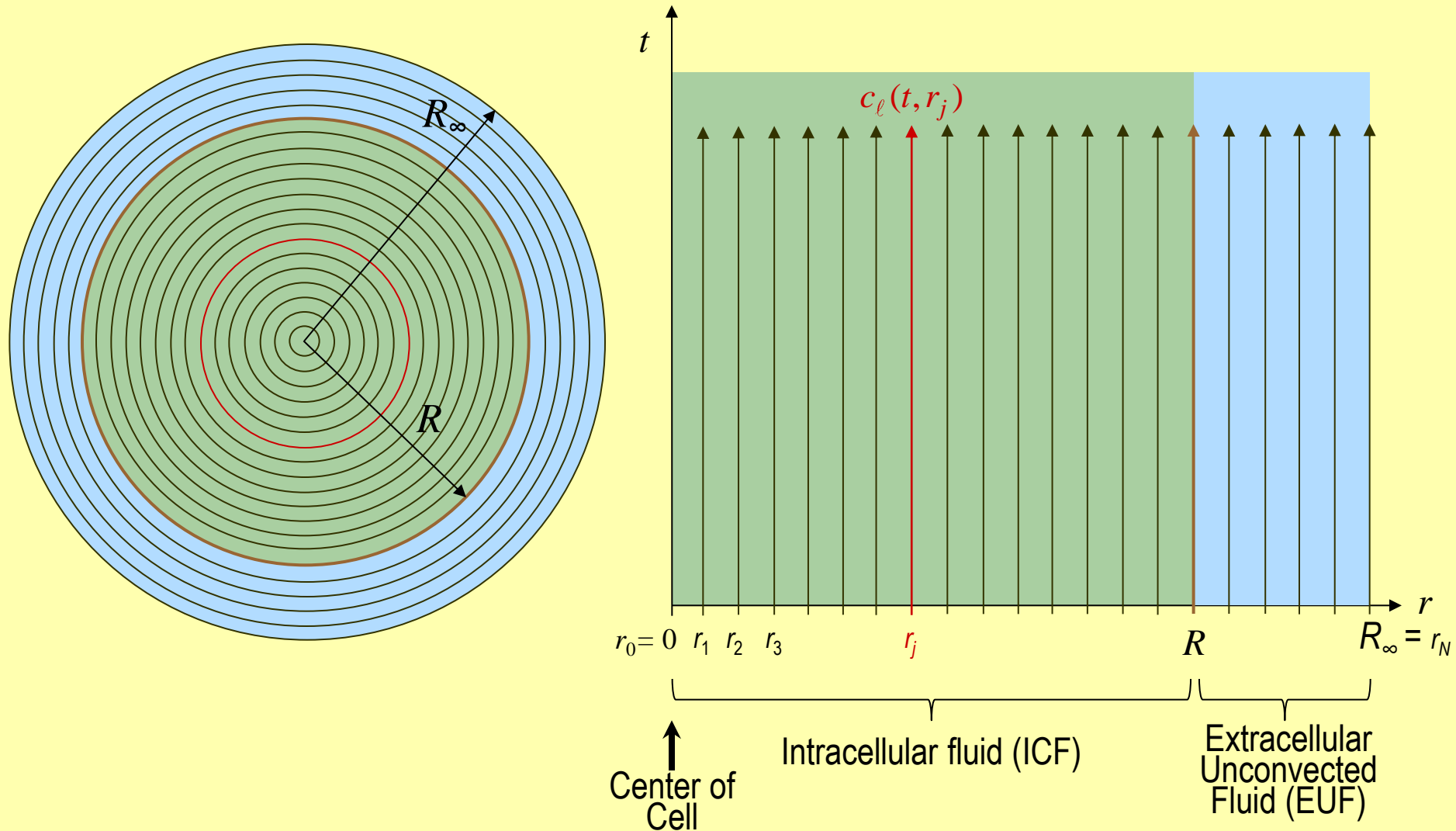
Assuming *spherical symmetry*, we write a reaction-diffusion equation for each species j ,

$$\frac{\partial}{\partial t} C_j(r, t) = \underbrace{\frac{1}{r^2} \frac{\partial}{\partial r} \left(D_j r^2 \frac{\partial}{\partial r} C_j(r, t) \right)}_{\substack{\text{Diffusion term} \\ \text{(Fick's second law)}}} + \underbrace{\sum_{\ell=-L-1}^{L+1} S_{j,\ell} \Phi_{\ell}(r, t)}_{\substack{\text{Reaction term} \\ \text{(law of mass action)}}, \quad 0 \leq r \leq R \leq R_{\infty},$$

with r distance from the center of the oocyte



Method of Lines



Numerical Experiments

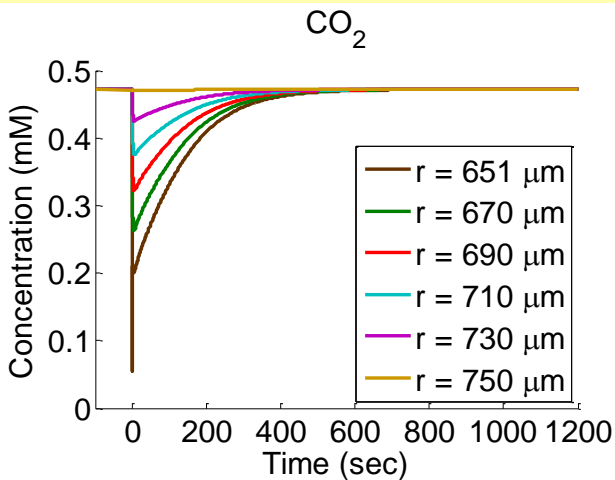
Assumptions

- The BECF, EUF, ICF and plasma membrane have same properties as water
- The EUF has thickness $d = 100 \mu\text{m}$
- Small CA-like activity uniformly distributed inside the oocyte and on the surface of the plasma membrane
- The BECF and EUF
 - contain 1.5% CO_2 /9.9 mM HCO_3^- / pH 7.50
 - have a single *mobile* non- CO_2 / HCO_3^- buffer with $\text{pK} = 7.5$ (e.g., HEPES) and $[\text{TA}] = 5\text{mM}$
- The ICF
 - has initial $\text{pH}_i = 7.20$
 - $[\text{CO}_2] = [\text{H}_2\text{CO}_3] = [\text{HCO}_3^-] = 0 \text{ mM}$
 - has a single *mobile* non- CO_2 / HCO_3^- buffer with $\text{pK} = 7.10$ and $[\text{TA}] \approx 27.31\text{mM}$

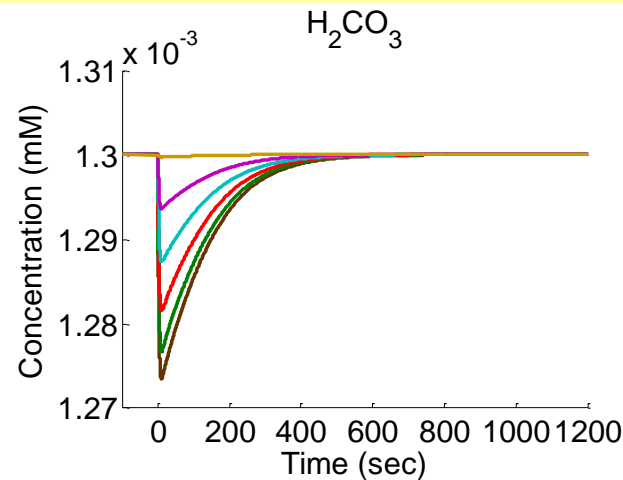
Results

Extracellular concentration-time profiles for solutes $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$

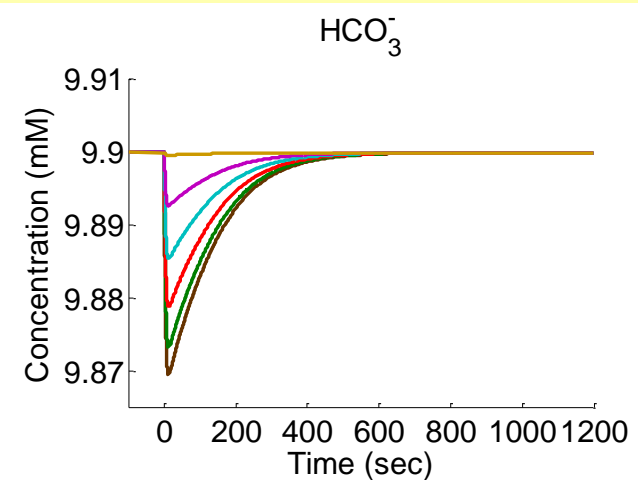
(A)



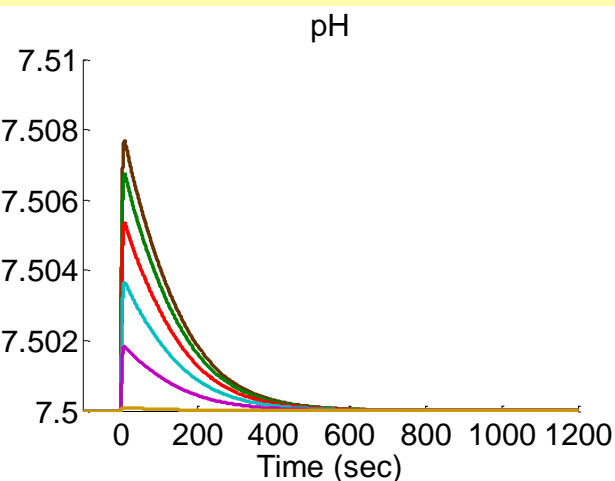
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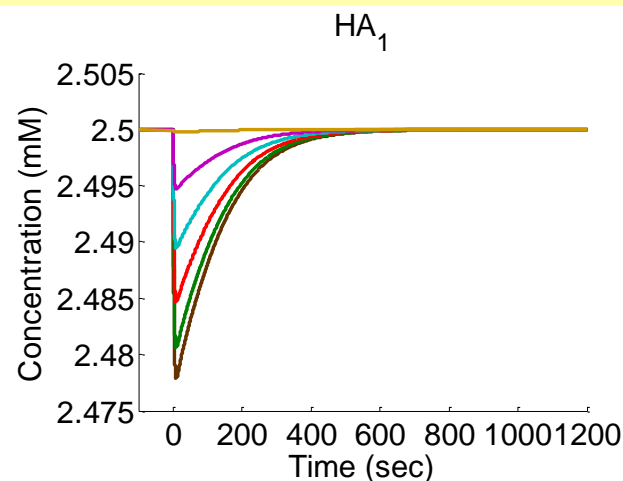
(C)



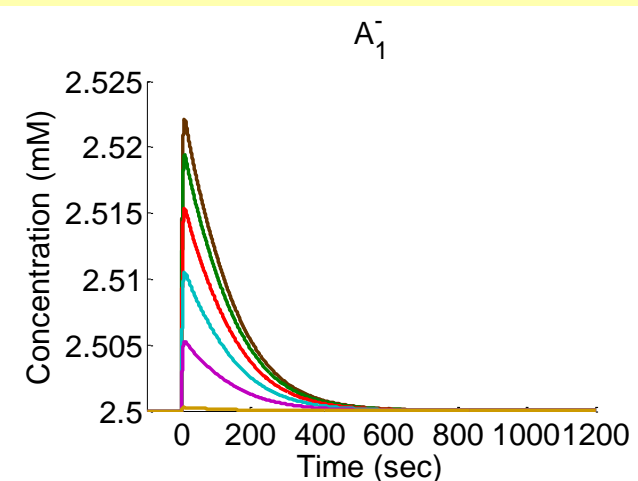
(D)

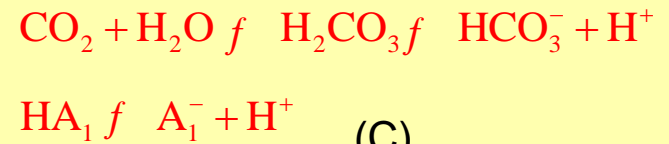


(E)

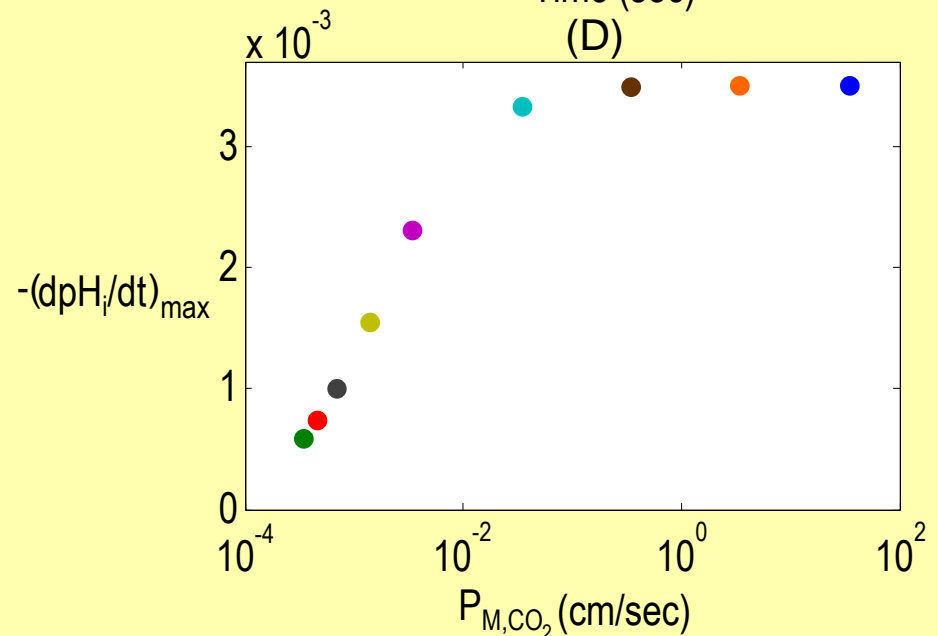
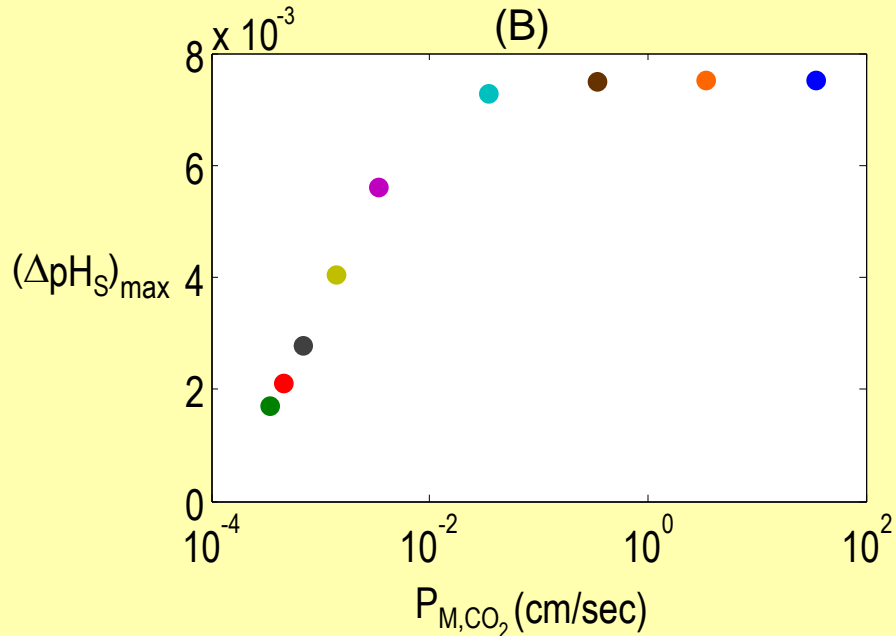
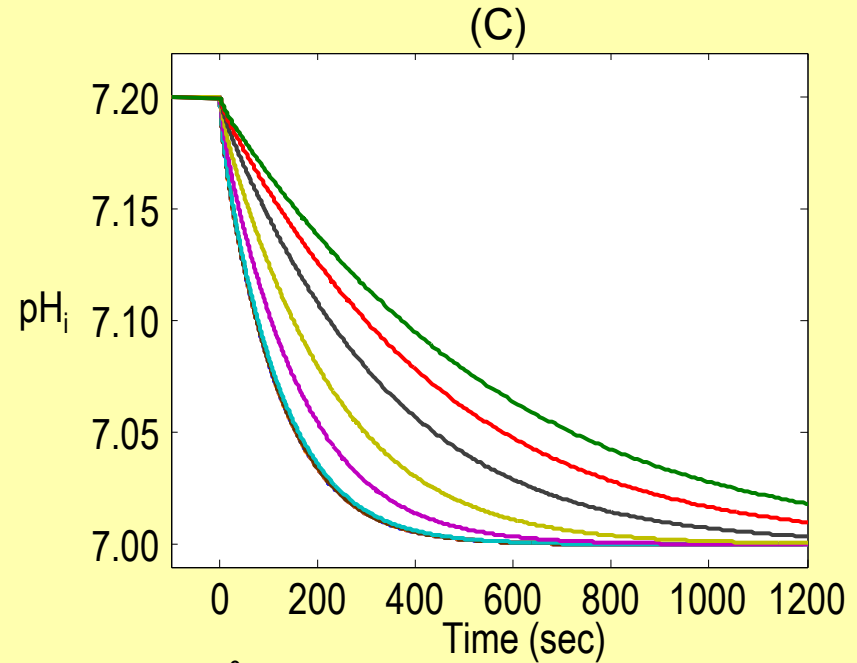
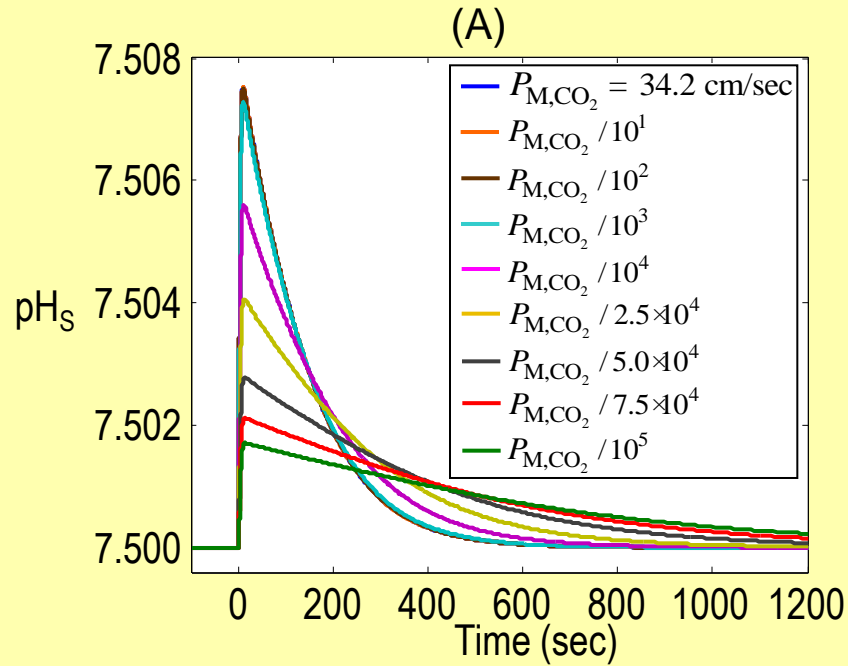


(F)



$$\begin{aligned} \text{CO}_2 + \text{H}_2\text{O} &\rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \\ \text{HA}_1 &\rightleftharpoons \text{A}_1^- + \text{H}^+ \end{aligned} \quad (\text{C})$$


Effects of Decreasing CO₂ Membrane Permeability



Implications

The background permeability of the membrane (i.e., in the absence of gas channels) must be very low

Given a sufficiently small P_{M,CO_2} , gas channels could contribute to CO_2 permeability even in the presence of a large d (in our numerical experiments $d = 100\mu m$)

With additional refinements to the model, we ought to be able to estimate absolute permeabilities

Effects of Changing the Width of the EUF

The EUF is a generalization of the concept of unstirred layer (UL)

ULs are thin, diffuse layers of fluid, always present near the surface of solid bodies immersed in a fluid, where molecules move predominantly via diffusion (Dainty and House, *J Physiol*, 1966; Korjamo et al, *J Pharm Sci*, 2009)

For a particular solute, the width of the UL (δ) is defined as

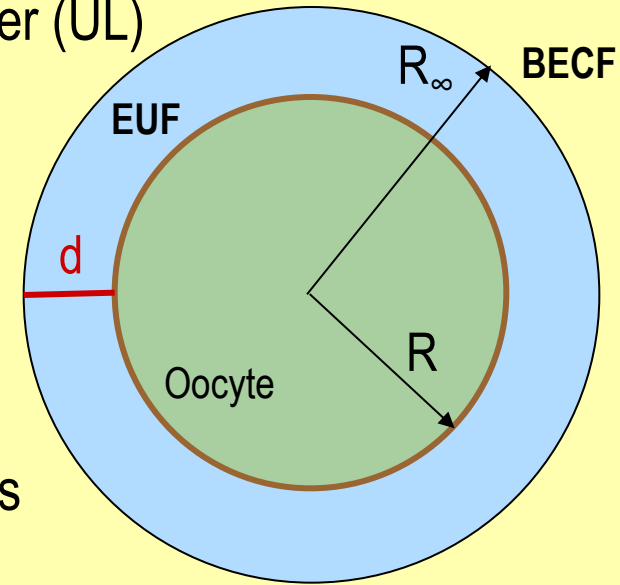
$$\delta = \frac{D}{P}$$

where D is the diffusion constant and P is the empirically measured permeability

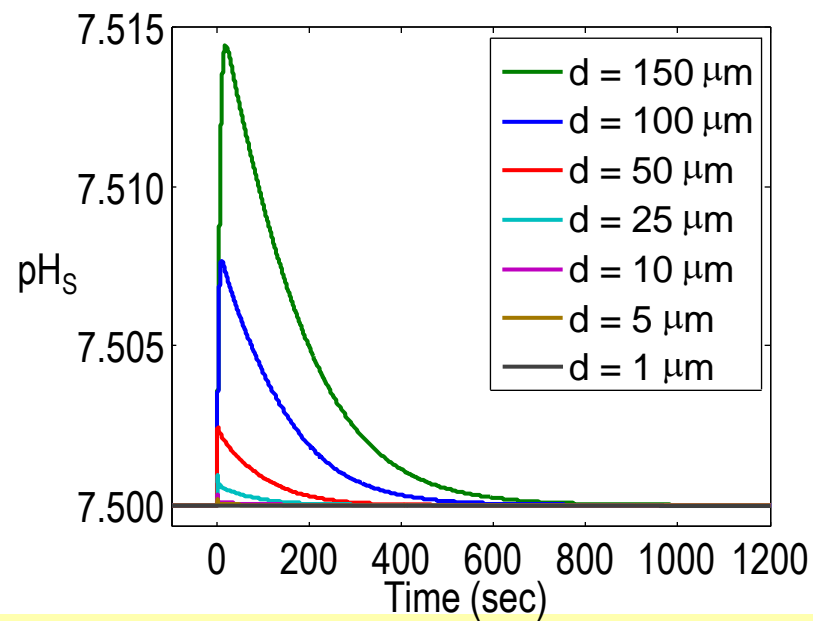
The width of the UL:

1. A steady-state concept
2. Solute-dependent
3. Ignores the effects of chemical reactions

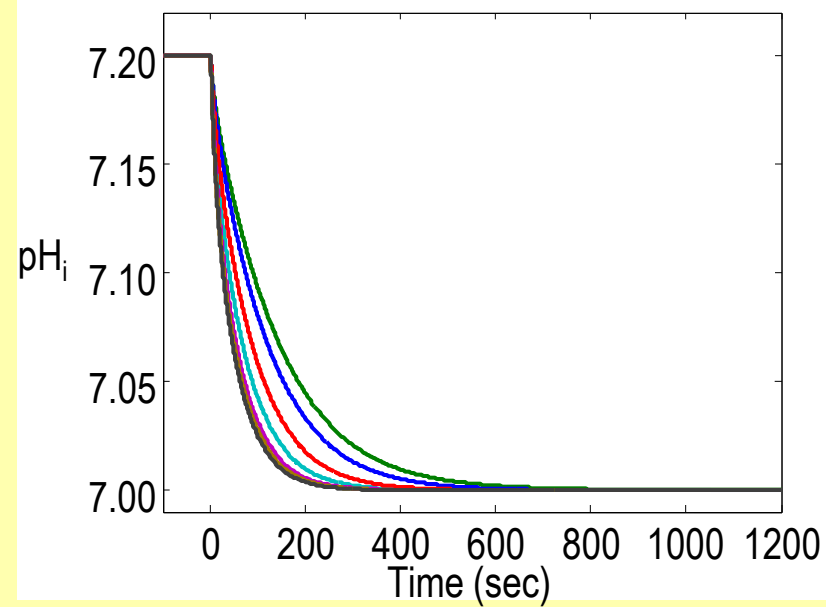
It is because our system is dynamic, involves multiples solutes, and solutes can react in the “UL”, that we decided to define the EUF



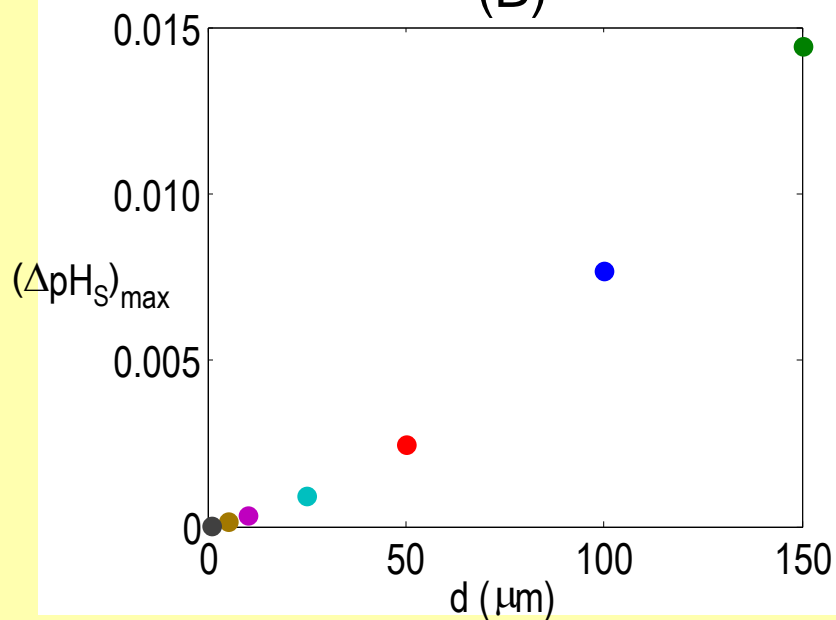
(A)



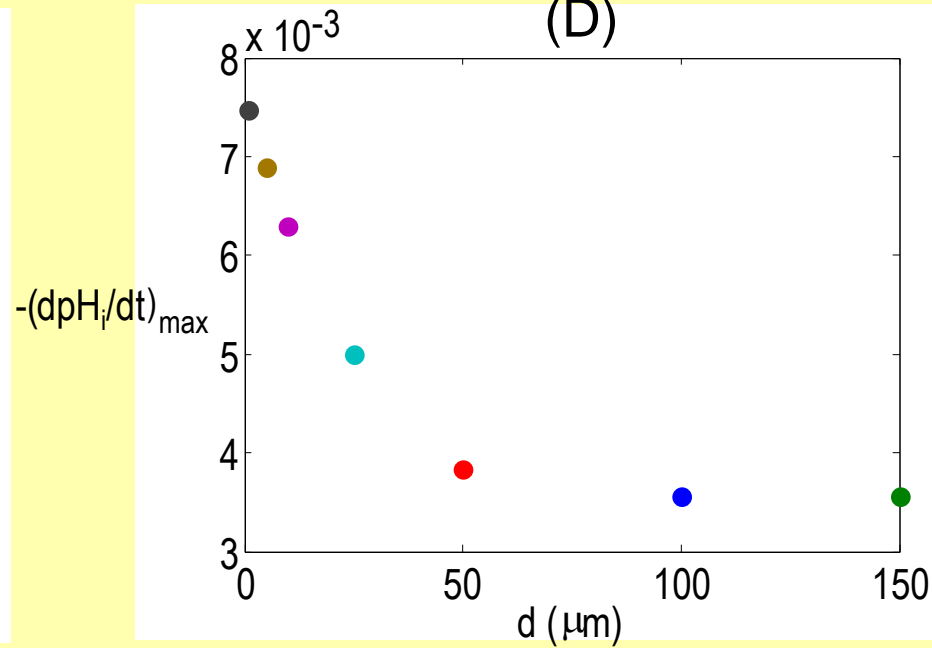
(C)



(B)



(D)



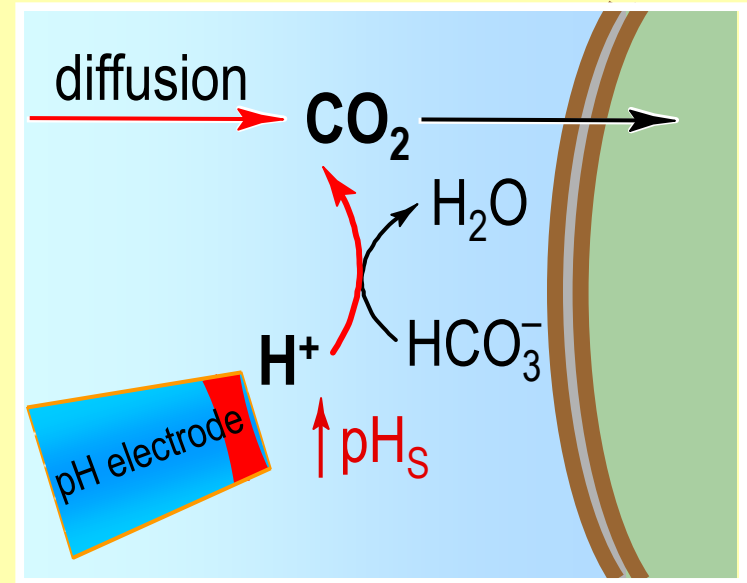
Implications

There is competition between diffusion and reaction in replenishing the lost CO_2 near the outer surface of the oocyte

We quantify this competition by introducing the diffusion reaction ratio (DRR)

$$\text{DRR} = \frac{\text{rate of } \text{CO}_2 \text{ replenished by diffusion}}{\text{rate of } \text{CO}_2 \text{ produced by reaction}}$$

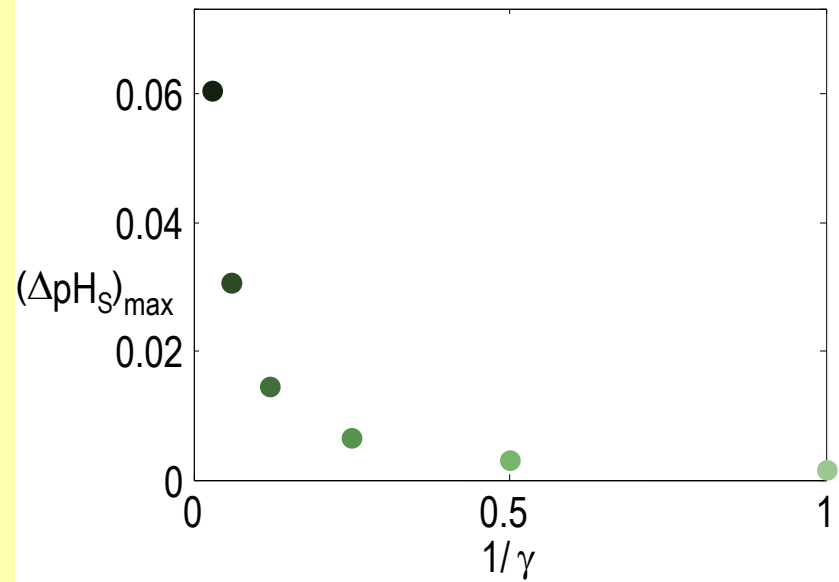
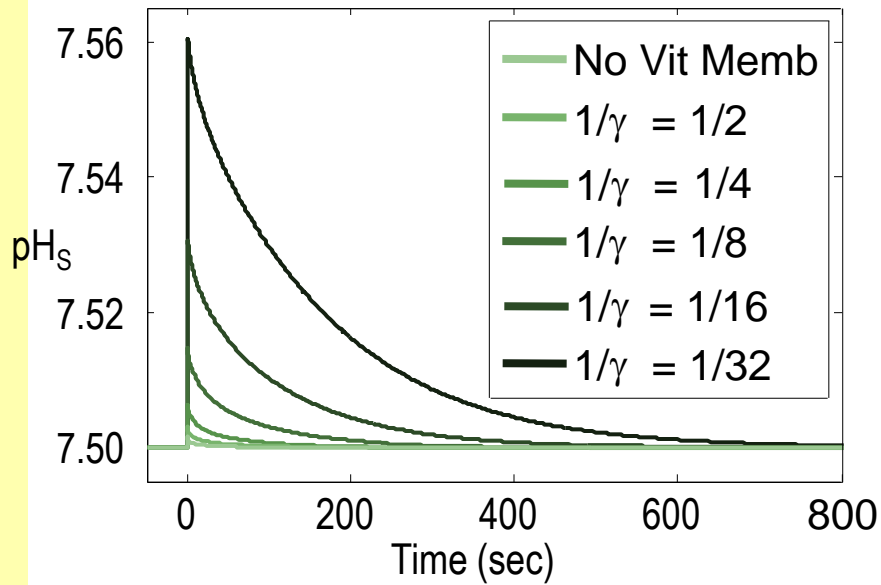
DRR rises as the width d of the EUF decreases



The Vitelline Membrane: pH_s Spike

Additional diffusion barrier to the movement of solutes

Implemented by reducing the mobility D of each solute near the outer surface of the oocyte by the same factor γ , i.e., $D_* = D/\gamma$



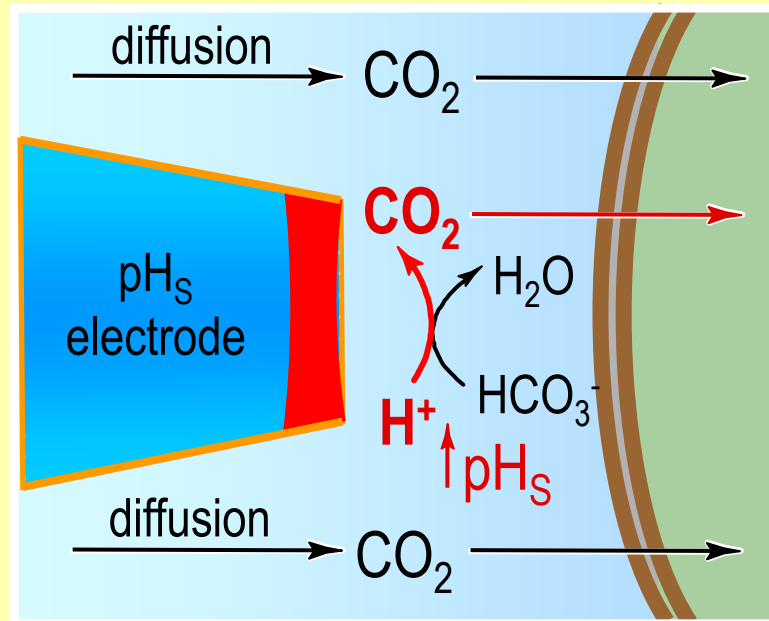
As we increase γ , the maximal height of the pH_S spike, $(\Delta pH_S)_{\max}$, increases

Implementation of the vitelline membrane reduces the contribution of diffusion and enhances the contribution of reaction at the surface

Implications

Implementation of the vitelline membrane – which reduces the contribution of diffusion and enhances the contribution of the reaction – can explain the height of the pH_S spike

Because the pH_S electrode creates a special environment with restricted diffusion, our implementation of the vitelline membrane somehow mimics this environment



Conclusions

The model can reproduce the pH transients observed experimentally

The simulations predict that:

1. The background permeability of the oocyte membrane must be very low
2. Given a sufficiently small P_{M,CO_2} , gas channels could contribute to CO_2 permeability even with a large EUF

The model provides new insights into the competition between diffusion and reaction processes near the outer surface of the plasma membrane

Future Directions

Apply the model to investigate the movements of ammonia and ammonium across the plasma membrane

Model the pH_S electrode's touching on the oocyte surface to explore the special environment underneath the pH_S electrode

Acknowledgments

Principal Investigator

Walter F. Boron, M.D., Ph.D.

Collaborators

Erkki Somersalo, Ph. D. (CWRU)

Daniela Calvetti, Ph. D. (CWRU)

Raif Musa-Aziz, Ph.D. (University of Sao Paulo)



9/6/12 @ 9:10 AM
Gas Channels Workshp
Emad
MD ... Sub-angstrom resolution

2016: Blue Waters \rightarrow 200K processors
 \neq

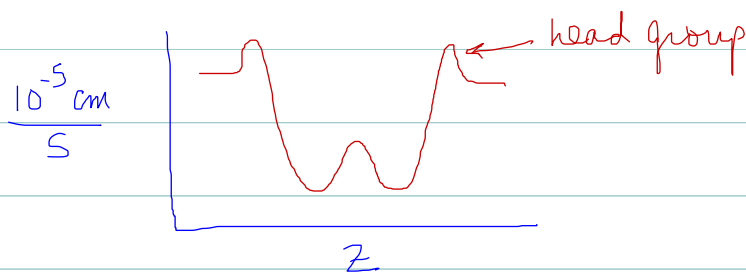
POPE 100%.

Start w/ ≈ 100 CO_2 near membrane

Modeling 50% Chol. is not trivial... where to place them, equilibration... have a partial solution.

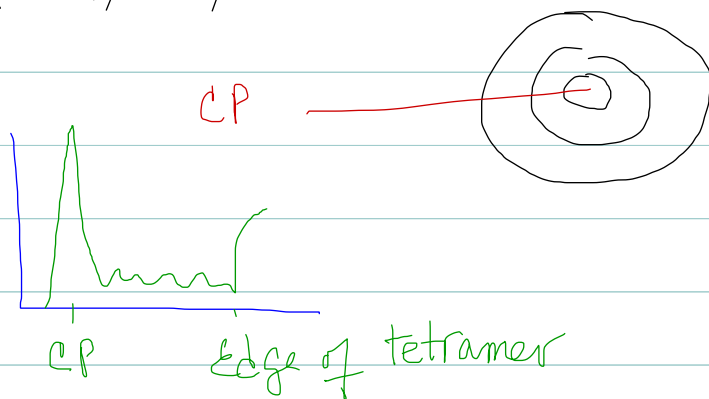
His partition coeff is \sim exp. det. values

Gas reaches equil in lipid in 10-15 ns for O_2 & CO_2 .



Implicit ligand sampling: Works \approx neutral molecules (not ions, wh. perturbs protein) ... results are about same as explicit.

{ AQPI, 4, 5
 { CO₂, O₂, NO



Transport through Aquaporins

SYSTEM	TOTAL (100x100 Å ²)	WATER PORES (4)	CENTRAL PORE (1)
Equi POPE-CO ₂	3	N/A	N/A
Equi POPC-CO ₂	5	N/A	N/A
Equi POPC-O ₂	16	N/A	N/A
Equi POPE-O ₂	11	N/A	N/A
Press POPE-CO ₂	168	N/A	N/A
Press POPC-CO ₂	160	N/A	N/A
Press POPE-O ₂	310	N/A	N/A
Press POPC-O ₂	208	N/A	N/A
Press POPE-AQPI-CO ₂	76	6	4
Press POPE-AQPI-O ₂	79	1	5

Two molecular simulation snapshots of a lipid bilayer. The left snapshot shows a standard lipid bilayer with green and red atoms. The right snapshot shows a lipid bilayer with a central pore, highlighted by a yellow and blue region.

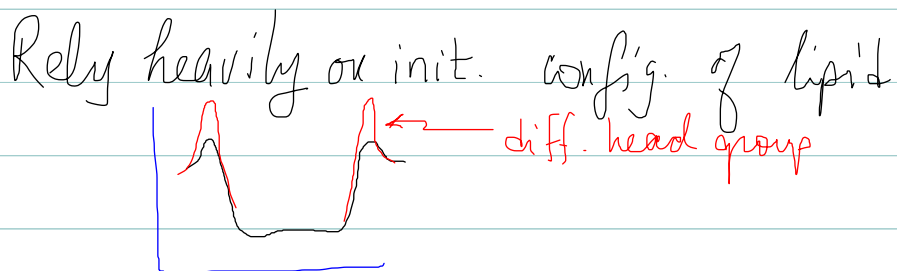
AQP1: D₅₀ → H₂O coord → major barrier

NO[•] through AQP4

O₂ " "
(Wang, Protein, 2010)

WB: Is [O₂] in CP the
same as in bulk
gas phase?

AQP4 (vs. 1): diff ΔG profile



Problem: lipid molec. move v. slowly!
10³ slower than H₂O

HMMM: highly mobile membrane mimetic
(liquid center of membrane)

Water-Oil attracts lipids to interface

Lipids are far more mobile

Even can see insertion of a peptide helix.

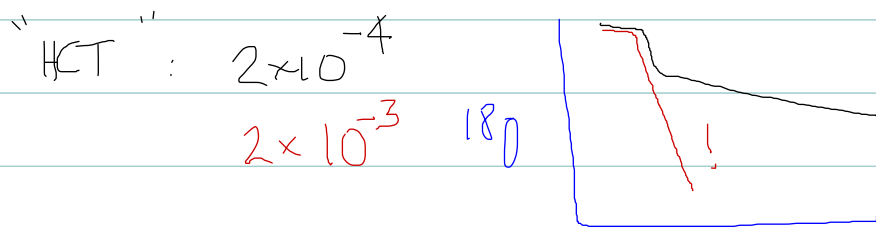
Chol might partition into core of bilayer,
parallel to plane of membrane.

9/6/12 @ 10:25 AM
Gas Channels Workshp
Gerolf Gros, Hannover
18O ... CO2 permeability

Problem is stopped-flow applied to vesicles

$t_{1/2}$ of CO₂ uptake by human RBC : 13 ms
O₂ 80

Measure ¹⁸O-labelled CO₂
46 vs 44 Mol. Mass



3-D curve fitting →
optimal P_{CO_2} & P_{HCO_3} ... no local minima

Critical params: A_i & pH_e ...
errors → big ΔP_{CO_2}
 $pH: \pm 0.01 \rightarrow 20-30\%$

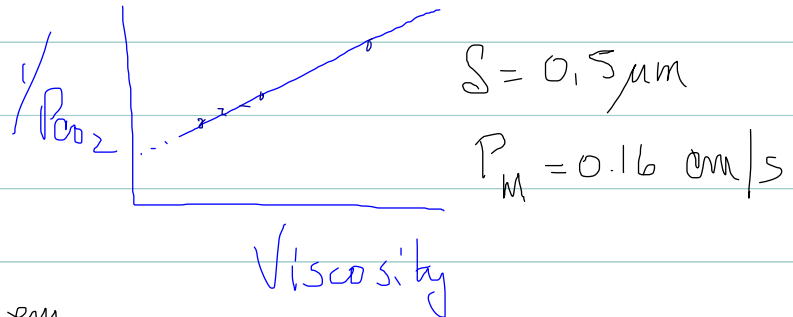
pH_i & P_{H_2O} are not important

This could be too fast, esp. if there is incomplete mixing.

But KO of AQPI + Rh could ↑ $t_{1/2}$ by 10x ...
make a Δ measurable?
WB

[illegible]

unstirred layer



\therefore UL is only
a minor problem
of α

P_{O_2} in human RBCs

$$\left. \begin{array}{l} \text{AQP1} \sim 50\% \\ \text{Rh} \sim 50\% \end{array} \right\} \sim 100\%$$

Colon:

IDEAS FOR DISC.

Competition
of H_2O vs. NH_3
or CO_2 through
aquapore

Conclusions

The ^{18}O exchange technique follows the decay of ^{18}O -labelled CO_2 in the extracellular fluid by mass spectrometry

This is possible because this decay is 1,000-10,000 times slower than net CO_2 uptake by cells or vesicles

The system of differential equations describing this process yields values of P_{CO_2} and $P_{\text{HCO}_3^-}$ from well defined minima of a fitting procedure

P_{CO_2} values can be determined over a range of 3-4 orders of magnitude

Parameters critical for calculation of P_{CO_2} and $P_{\text{HCO}_3^-}$ are intracellular CA activity and extracellular pH , both of which are carefully controlled

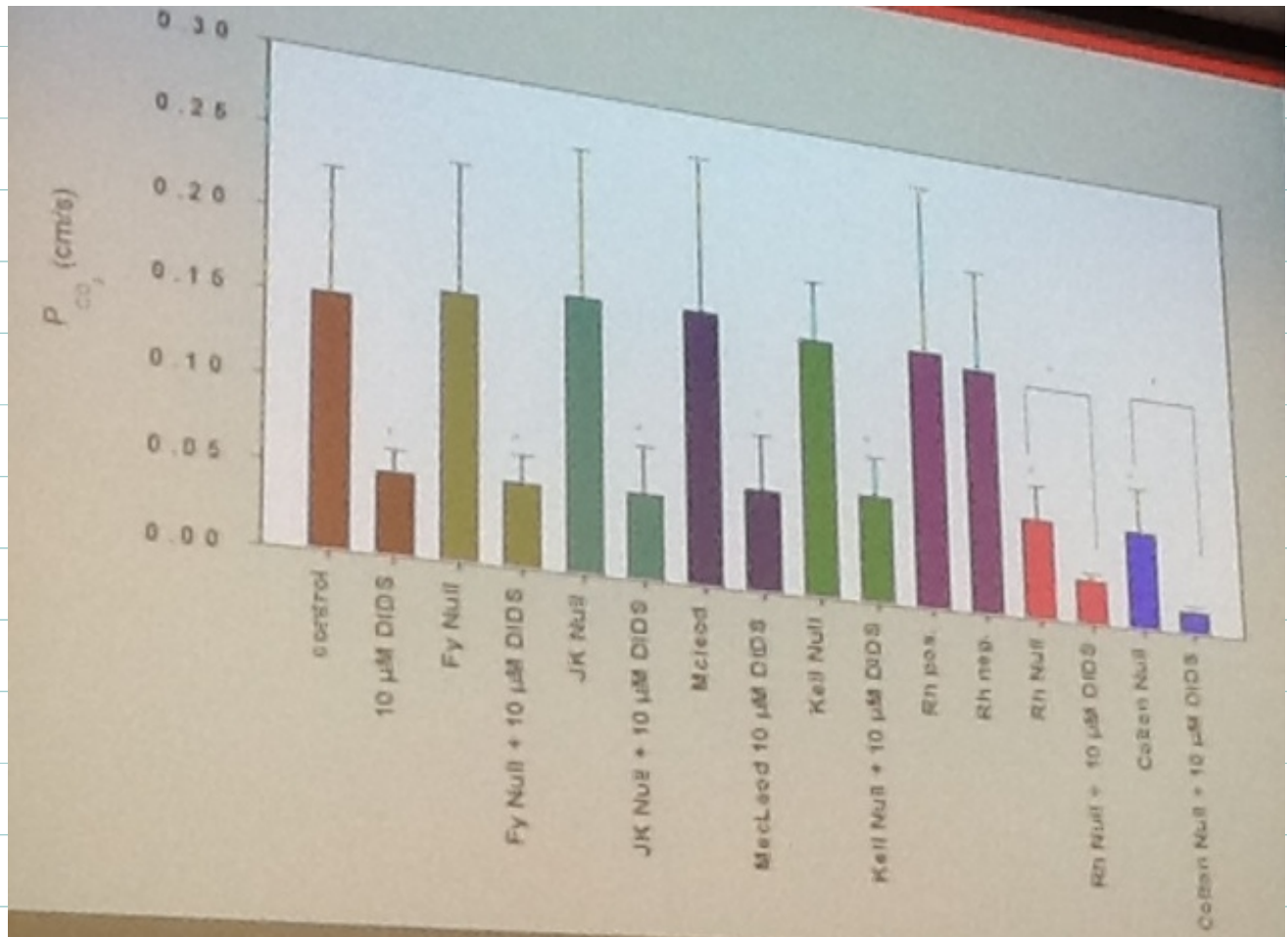
Unstirred layers affect the results by no more than ~ 25%

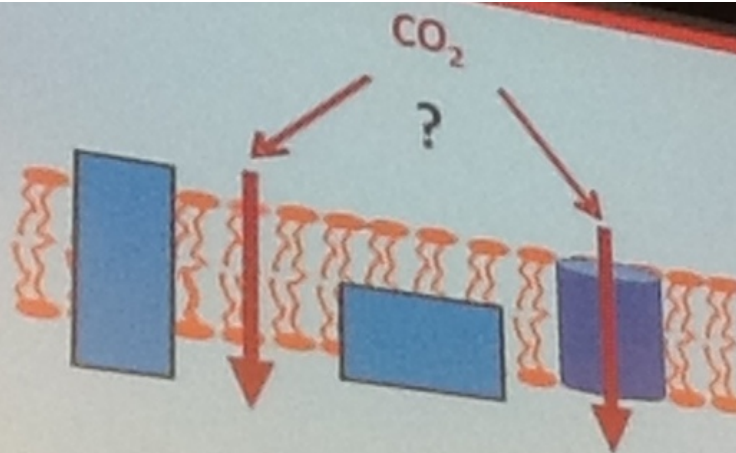
The method is applicable to suspensions of isolated cells or vesicles and to intact epithelia

$$[X]_w = S_w P_x$$

$$[X]_L = \frac{S_L}{S_w} \underbrace{[X]_w}_w = \frac{S_L}{S_w} \cdot \underbrace{S_w \cdot P_x}_w$$
$$= S_L \cdot P_x$$

9/6/12 @ 11:15 AM
 Gas Channels Worksop
 Volker Endeward, Hannover
 Background CO₂ permeability





1. What are the intrinsic CO₂

Cell membranes show CO₂ permeabilities lower than synthetic lipid bilayer

	$P_{\text{CO}_2} \text{ (cm/s)} \pm \text{S.D.}$
Synthetic lipid bilayer	0.35 / 3.2 ~1
Red cell, Ø functional gas channel	0.015 ± 0.003
MDCK	0.017 ± 0.004
tsA201	0.007 ± 0.003
Basolateral membrane of proximal colon epithelium	~0.022
Apical membrane of proximal colon epithelium	0.0015 ± 0.0006

P_{CO_2} (art. lipid bilayer) \gg naked mammalian membrane

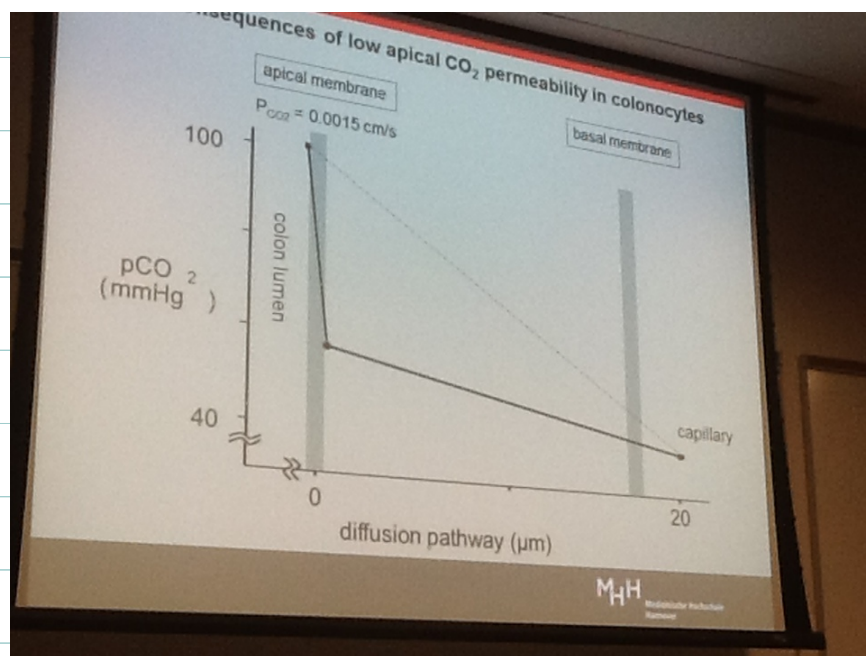
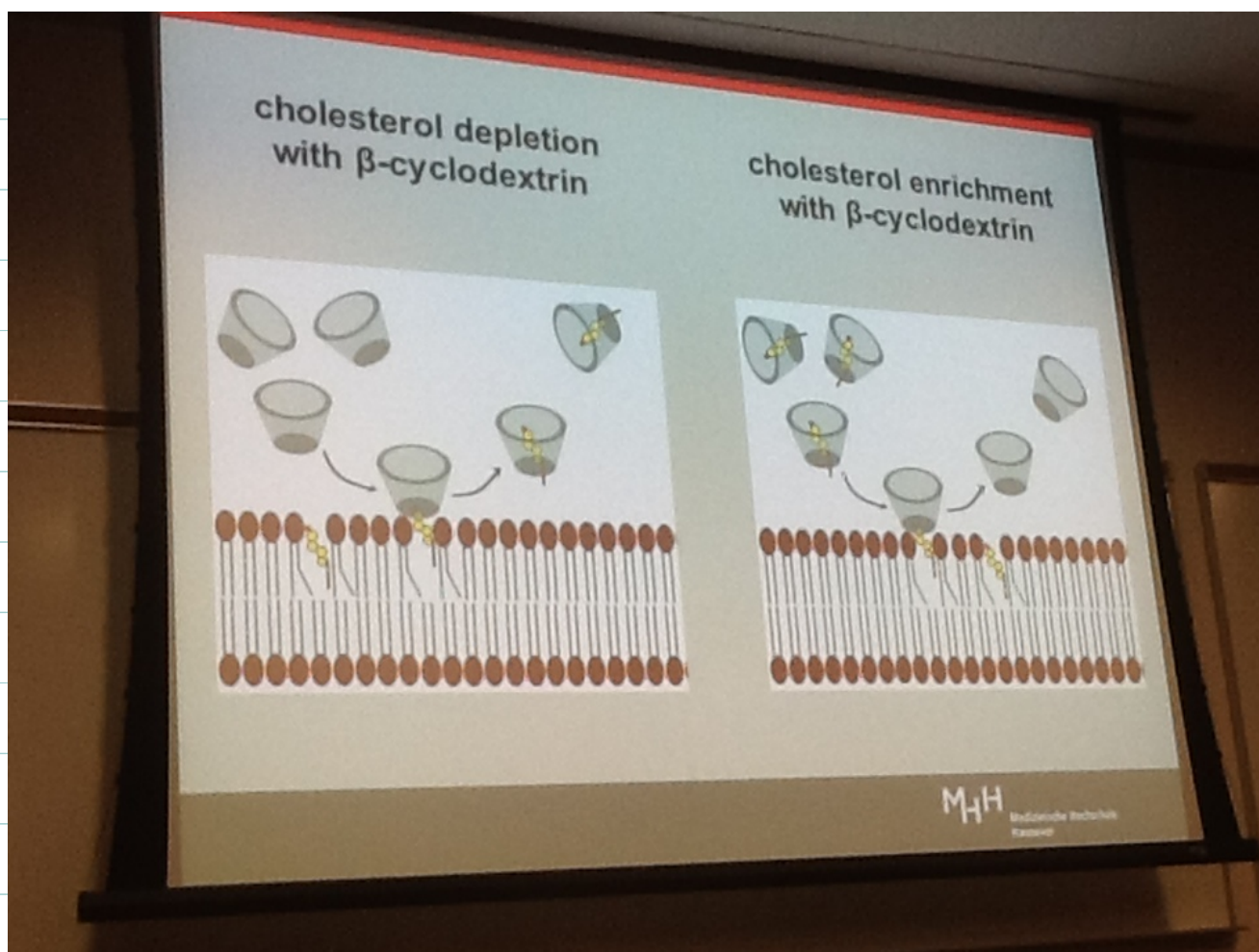
Cholesterol: 98% \downarrow in P_f .
 \neq

150 nm: mean vesicle diam... contain CA II

Chol: 0-20% \rightarrow \nexists measurable
30-70% \rightarrow log-linear \downarrow P_{CO_2} by $\sim 10^2$

Apical colonic membrane: 70% cholesterol

β -cyclodextrin



Could we $\downarrow P_{\text{CO}_2}$
of X_0 by \uparrow
chol &
Vice versa.

- From these considerations we can see that gas exchange of cells with a low CO_2 permeability is limited
- Hypothesis: cell membranes with normal cholesterol and low intrinsic P_{CO_2} adapt their CO_2 permeabilities to their needs by incorporating gas channels in the membrane.

AQP1 vs. Agpz in liposomes
 $\uparrow\uparrow P_{\text{CO}_2}$ $\uparrow \Delta P_{\text{CO}_2}$

He sees a much bigger effect (? ~80%) than we do.

Gas	CO_2	O_2	NO	N_2
Lipid-water partition coefficient	0.95	2.9	3.8	4.1
Permeability (phospholipid membrane)	~1 cm/s	~3 cm/s	~4 cm/s	~4 cm/s

O_2 : PL membrane 3 cm/s

chol: 1/100

PL + Chol: 0.03?

Heart m : $\Delta p_{CO_2} = 40$ mmHg

way too high to be
physiol. possible.

Summary

With rising cholesterol content the CO_2 permeability (P_{CO_2}) of lipid vesicles decreases drastically.

The intrinsic P_{CO_2} of cell membranes is low due to their cholesterol content:

- 1) cell membranes and lipid vesicles with identical cholesterol content exhibit identical CO_2 permeability
- 2) cholesterol-depleted cell membranes have an increased CO_2 permeability, cholesterol-enriched cell membranes a reduced permeability

Cell membranes with normal cholesterol and low P_{CO_2} raise their CO_2 permeability, when functionally required, by incorporation of CO_2 channels.

- 1) AQP1 incorporated in lipid vesicles raises CO_2 permeability in a concentration-dependent manner
- 2) AQP1 expression in MDCK cells increases membrane P_{CO_2} .

$$P_{CO_2} \propto [AQP]^n ?$$

9/6/12 @ 1:05 PM
Gas Channels Worksoop
Bhanu Jena, Wayne State
Cholesterol

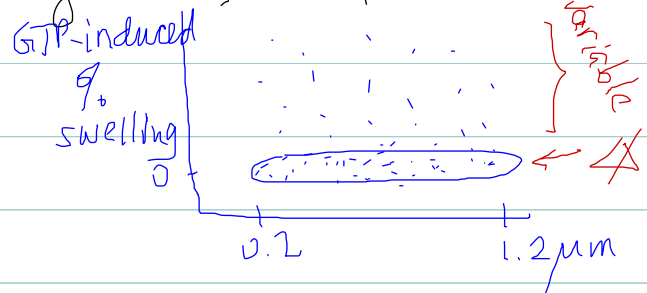
Got interested in AQPs because of their involvement in vesicle fusion.

H_2O must enter the vesicle

$G\alpha_{13}$: assoc. \bar{c} ZSM

GTP \rightarrow \uparrow water by volume
(AFM) $\nless 3H_2O$.

Jena et al, PNAS, 1997

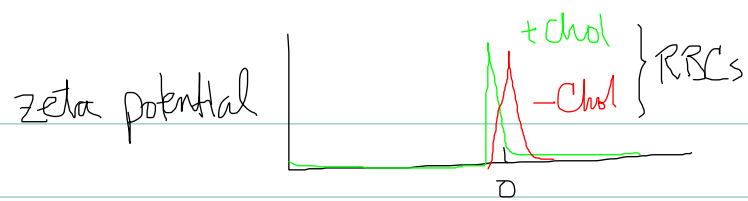


AQP1: Swelling complex } not 2,3,4,5,
" 6: " } 7,8,9

Mast (mastoparan)

swelling
 $\% \Delta \text{Volume of granule}$: GTP + Mast
+ 20-40 μM M β CD (cyclodextrin)

AQP6: $G\alpha_o$, V-H⁺ ATPase ^{stability} complex req. Chol.
Remove Chol \rightarrow complex falls apart.

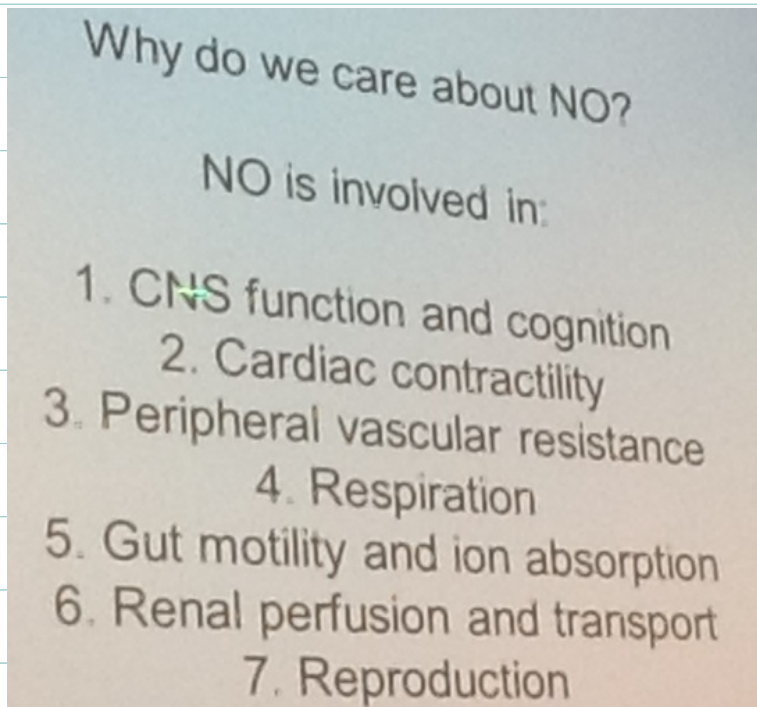


9/6/12 @ 1:55 PM

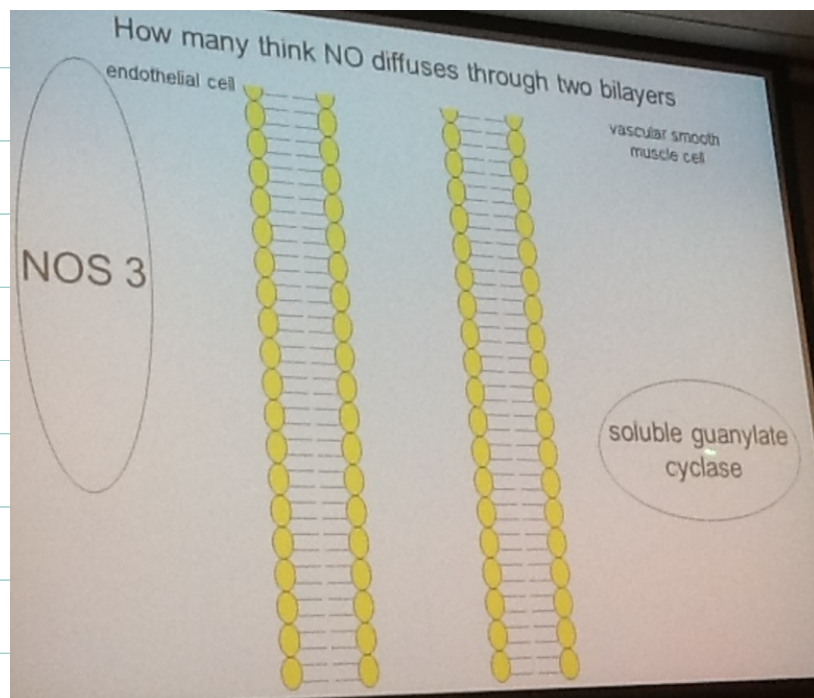
Gas Channels Workop

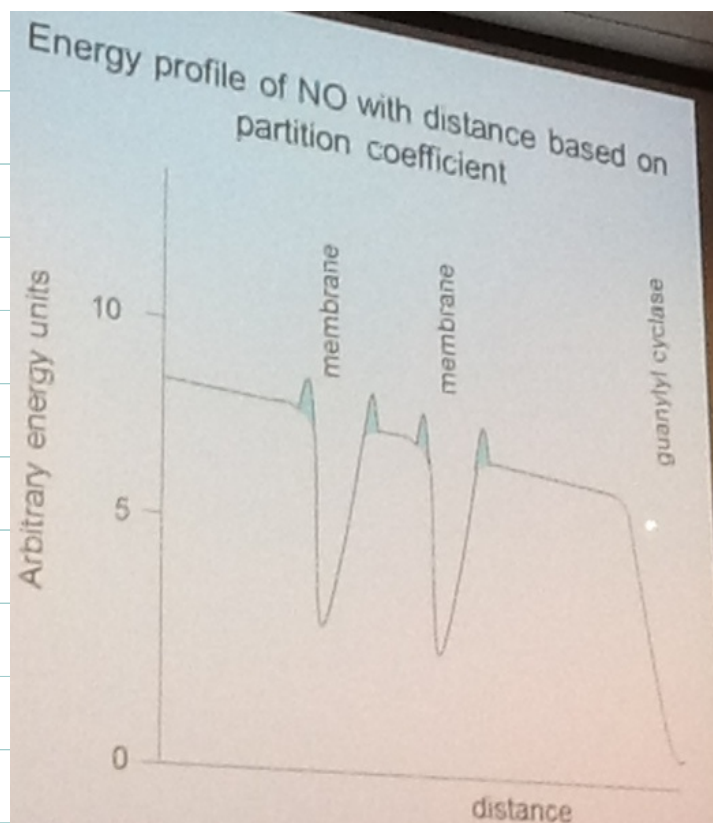
Jeff Garvin, HFH

The truth about the movement of NO across cell membranes



Partition coef: 3-5
 $t_{1/2} \sim 30 \text{ s}$





"Partition coeff say nothing about rates" ... his PhD mentor beat into him that S/S is an equilibrium parameter.

What is the chemistry of NO in lipid?
More or less stable than in H_2O ?

If our hypothesis is correct:

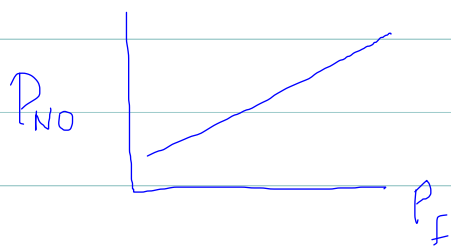
1. NO permeability (P_{NO}) should correlate with water permeability (P_f).

2. Increasing AQP-1 expression should increase NO flux.

3. Inhibitors of AQP-1 should reduce NO flux.

4. NO flux should be saturable.

5. Purified AQP-1 should transport NO.



P_{NO} : fluorescent probe
DAF2

NO influx \rightarrow CHO cells, transiently transfected.
NO: NO donor or gas.

Hg inhibitors \bar{c} NO gas?

$K_{1/2}$: $0.54 \mu M$ Physiol $[NO] \approx 0.2 \mu M$

AQP1 reconstr. into vesicles $\rightarrow \uparrow J_{NO}$

CHO cells:

AQP3: 25% \uparrow over mock \ll AQP1. Did not \checkmark expression.

" 4: 30% \uparrow ~~✓~~

Aortic ring: isometric force

PF = Phenylephrine \rightarrow contr.

Vary [ACh] to relax. AQP1 KO: \downarrow ACh response
? \downarrow NO efflux from EC or \downarrow influx into VSMC

KO: \downarrow NO release from EC

\downarrow " uptake into VSMC

- Conclusion
1. AQP-1 transports NO.
 2. Transport of NO by AQP-1 occurs faster than by diffusion through the bilayer by about a factor of 2.
 3. Transport of NO by AQP-1 appears to be physiologically significant.
 4. Reduced ACh-dependent relaxation of aortic rings from AQP-1 $-/-$ mice is due to both reduced efflux out of endothelial cells and reduced influx into vascular smooth muscle cells.

32 in Audience

9/6/12 @ 3:10 PM

Gas Channels Workop

David Weiner (wee)

Assessing roles of Rh glycoproteins in NH_3 gas transport?

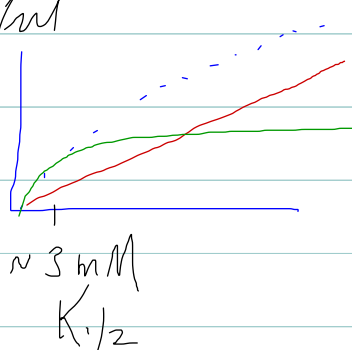
Is NH_3 transport "diffusive" or protein mediated?

Inhibitors: none

si RNA: unsuccessful

Saturation?

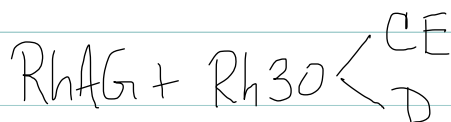
Me- NH_2
Basolat.
uptake



Saturable component +
diffusive comp.

Diffusion may dominate
in inner medulla,

where $[\text{NH}_3]$ is high & Rh levels are \uparrow low.



Cannot find RhAG ^{by Ab} anywhere but RBC.

1st cloned: RhAG

#2

"

BG: Perivascular cells in hepatocytes
Hair follicles. $[\text{NH}_3]$ is
 $100\times >$ plasma. Goes \uparrow
Exercise: $2 \rightarrow 10 \text{ mM}$.
Urine: $200-300 \text{ mM}$
(higher NH_3 conc)

GI \rightarrow NH_3 250 mmole/day. SI > Colon

Lungs: RhBG \rightarrow not in alveolar cells but in
Bronchial epith cells.

RhCG: ? Glu neurotransmission.

Liver: $[\text{NH}_3]$ is \uparrow in bile

Muscle: Exercise \rightarrow 4-5x NH_3 ... produced
by sk.m. At rest, sk.m. is a
 NH_3 sink. excreted

Kidney: 1-2% of NH_3 from GFR.

CD: RhBG: BLM \swarrow BalbC
CG: AM + some BLM \leftarrow
C57 BL6: much higher

MAc \rightarrow \uparrow RhBG expression

Does Tenidap \downarrow the
"CO₂ permeability"
attributable to NBC?
Does it speed up pH_i \downarrow ?

Grant: Mutant NBCe1
{ Cond. KO?
{ Mutations

Conditions where Rhbg and/or Rhcg expression parallels ammonia transport

- **Metabolic acidosis**
 - Seshadri RM, et al, *AJP Renal* 290: F397-408, 2006.
 - Seshadri RM, et al, *AJP Renal* 290: F1443-52, 2006.
 - JM Bishop, et al, *AJP Renal* 299: F1067-77, 2010.
- **Reduced renal mass**
 - HY Kim, et al, *AJP Renal* 293: F1238-F1247, 2007.
- **Ischemia-reperfusion injury**
 - KH Han, et al, *AJP Renal* 293: F1342-F1354, 2007.
- **Cyclosporine A-induced renal tubular acidosis**
 - SW Lim, et al, *Nephron Exp Nephrology* 110: e49-58, 2008.
- **Hypokalemia**
 - KH Han, et al, *AJP Renal* 301: F823-F832, 2011.
- **Adaptive response to deletion of other acid-base transporters**
 - **Pendrin**
 - Kim YH, et al, *AJP Renal* 289: F1262-F1271, 2005.
 - **Collecting duct Rhcg**
 - HW Lee, et al, *AJP Renal* 296: F1364-F1376, 2009.
 - **Intercalated cell-specific Rhcg**
 - HW Lee, et al, *AJP Renal* 299: F1369-F1379, 2010.
 - **Intercalated cell-specific Rhbg**
 - JM Bishop, et al, *AJP Renal* 299: F1065-F1077, 2010.

Conditional KO of Rhcg : ↓ urinary excretion but
 Δ pH_a

Slowest renal response : NH₃ transport ... req. 4-5 days

Our studies assessing the role of Rh glycoproteins in NH_3 gas transport

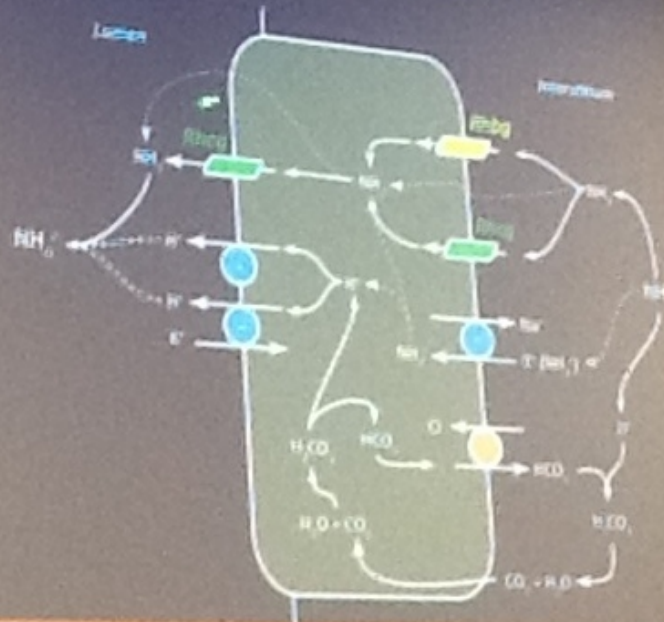
Renal collecting duct NH_3 transport is both diffusive and saturable

Rh glycoproteins are present specifically in cells that transport NH_3

Rh glycoprotein expression parallels NH_3 gas transport

Rh glycoprotein gene deletion alter NH_3 gas transport

Rh glycoprotein-mediated NH_3 transport is central to renal ammonia metabolism and transport



9/6/12 @ 4:00 PM

Gas Channels Workop

Robert Stroud, UCSF

What do structures tell us about Gas Channels? QED!

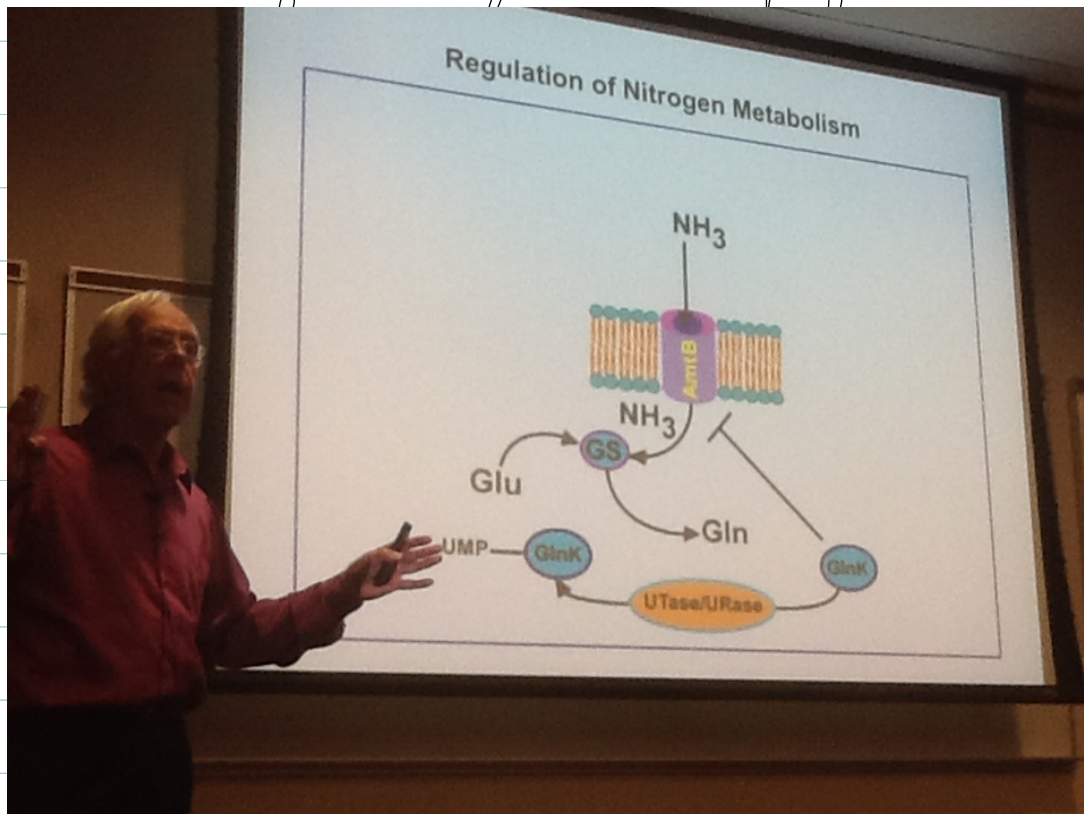
Much harder to discover channels for neutral substances (H_2O , 1996 ... gases only now).

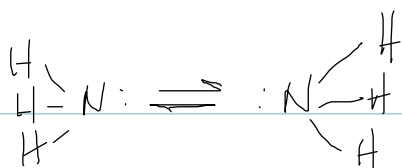
QED: Quantum Electrodynamics

I. Rh Family (Amt/MEP)

Bact. Yeast

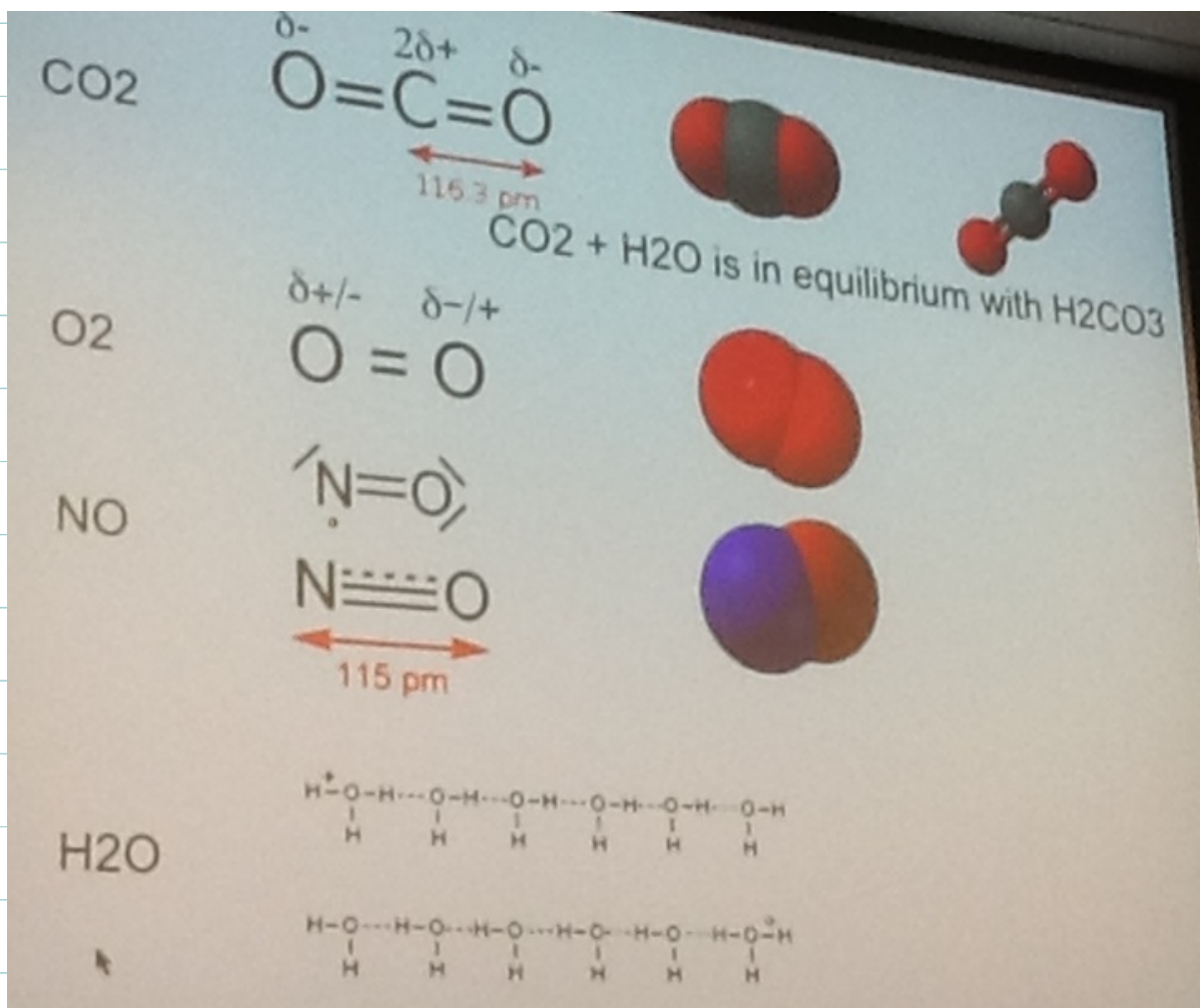
Bact: reg. N as food source, pref. as NH_3





Rapid inversion

Dipole moment $\sim \text{H}_2\text{O}$



Why is H₂O not a gas ... H-bonding

Some still think that Rh prot.
are NH₄⁺ channels!

But H₂O can
be a gas! WB

AMB : 1.35 Å

11 TMs

inverted repeat

NH₃: black hole of crystallography
10 e⁻ also Na⁺, Mg²⁺

Can also use MeNH₃

Channel: No H₂O. 1/7 occupancy by NH₃ @ 3 sites.
pore

pKa 9.6 → < 7
binding of NH₄⁺

No water, no ions

Why important for Biology?



- K^+ channels;

An NH_4^+ channel could 'leak' K^+ and hence membrane potential in eukaryotes.

- Amt/MEP are impermeable to any other ions.

- NH_4^+ unstable at the centre of the hydrophobic bilayer while NH_3 is not. Cf K^+

- NH_3 versus NH_4^+ would not leak proton motive force in conduction.

- No energy nor counter ion is needed to accumulate ammonia.



AmtB

Completely turned off when enough NH_3 is around!

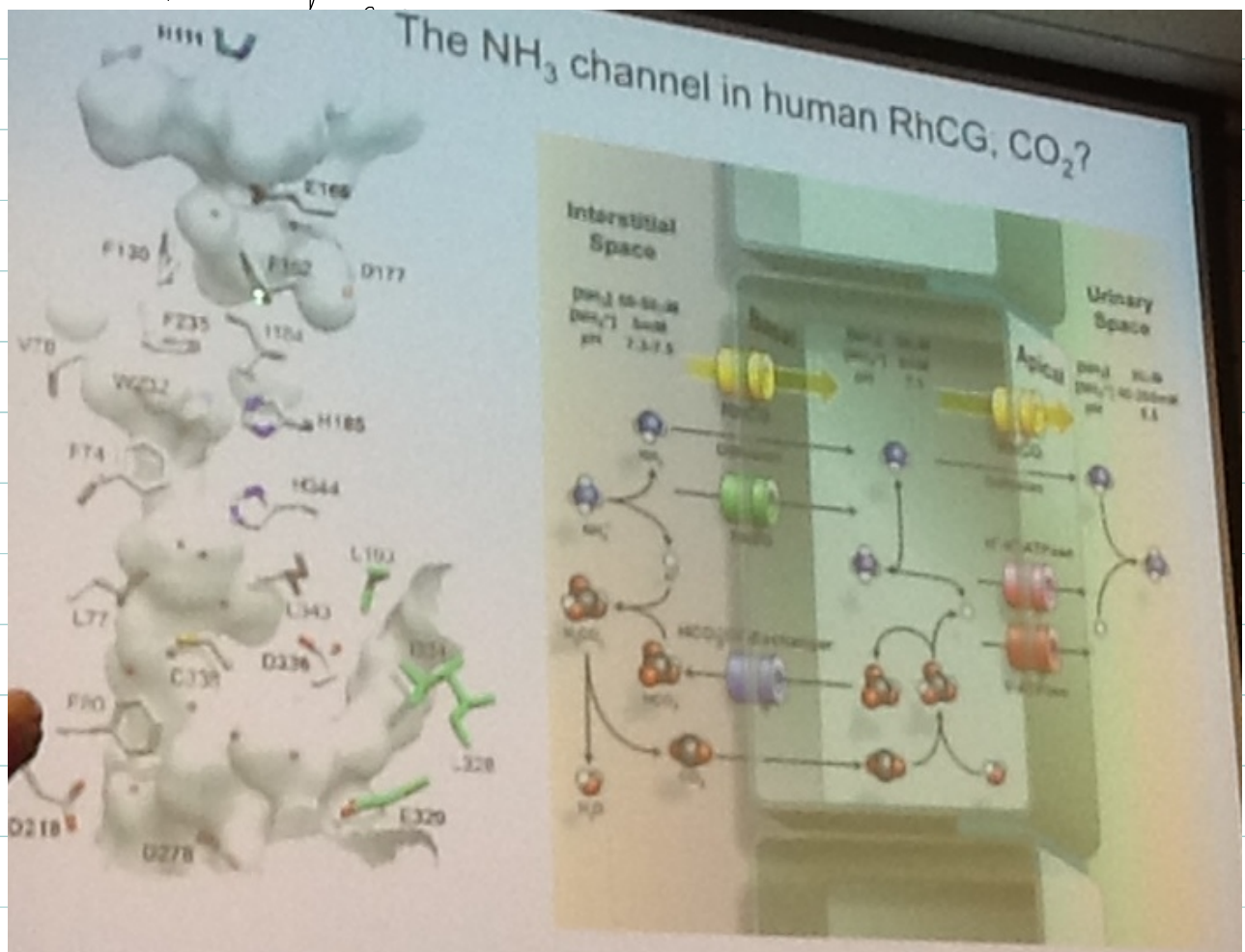
GlnK: Finger points to AmtB & blocks it.

ADP bound

Prevents reprotonation
of entering NH_3 .

Nitrosomas europea ... more similar than Amt to
mammalian Rh. Has a 'stalk'
extending into cytosol. (They do not
NeRh pay much attn. to it)
≠

RhCG: Expressed in HEK293s



"NIH Common Fund" 4th NIH Roadmap meeting
Nov. 28-30
SFO: Westin Hotel

II. Aquaporins

Pf AQP: Plasmodium \rightarrow Glycerol

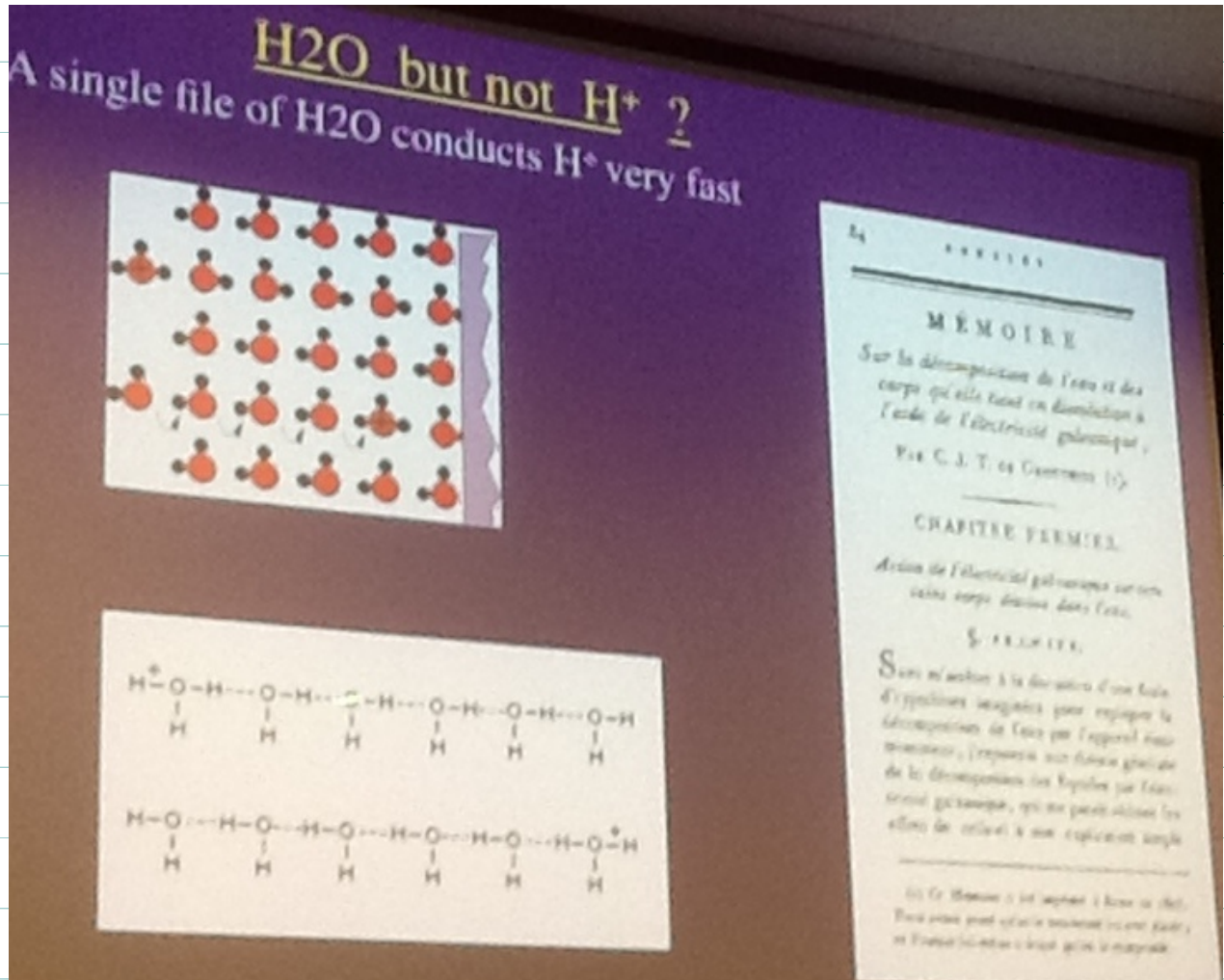
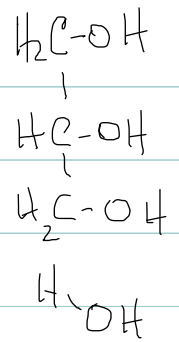
1.8 Å - Rotamers can be unambiguously determined

9 H₂O molec.
in a chain

- H₂O density clear

- Solutes can be identified

Glycerol is Water like



Beitz et al PNAS, 2004

An AQP that transport H_2O & Glycerol both v. well.

↖ Malaria

One mutation: Glu → Ser (way up above aquapore) → H_2O permeability.

↓
Removes 1 H bond.

Channel holds on to H_2O .

9/7/12 @ 8:30 AM
Gas Channels Workshp
Ryan Geyer
O2 Transport in RBCs

Ryan: What was sampling rate?

Dead time?

9 wavelengths: PMT, array?

Fumbling \bar{c} details cost you control of preten^{tation}.

Showing non-case KDs \bar{S} their controls was shooting yourself in the foot.

{ Q_{10} : ?

{ What was Verkman's Q_{10} for P_f ? 4?

CK MCV : Is there a slv Δ . CK Hb.

{ CK P_{50} in WT vs KO vs blockers.

{ 2, 3 DPG, pH, etc \bar{S} are addressed by P_{50} .

[WP interpretation: we'll be OK, but we have to dot
i's & cross t's to be sure that your Δ s
are not due to something other than the
cell membrane.]

9/7/12 @ 8:30 AM
Gas Channels Workshp
Rossana Occhipinti
Mathematical modeling

WB: Would be nice to have a dye to monitor p_Hs.

DRR: spelling error.

Jeff: Animal vs. Vegetal poles

Bhannu: Optical tweezers → viscosity across
the entire diameter.

9/7/12 @ 9:30 AM
Gas Channels Workso
Xue Qin
CO2 permeability of AQP5

Emad : Rotamer search ... what is stable?

Gas channels Workshop

September 6-7 2012

Lecture 1 : Walter Boron - Gas Channels

- Solubility theory
 $P \propto S_L / S_w$
Note: Henry's law is true at steady-state
- Solubility-Diffusion theory
- Access-Solubility-Diffusion-Egress theory

Lecture 2: Emod

Newtonian equations

Major limitation \rightarrow time scale (speed limit: 1fs)

Force field approximations

atomistic resolution

Implicit Ligand Sampling $W(r) = -k_B T \ln \left[\frac{P(r)}{P_0} \right]$

$$F(z) = -RT \ln \frac{\sum e^{-F(x,y,z)/RT}}{\dots}$$

Lecture 3: Gerolf Gros - Measuring CO_2 permeability by ^{18}O Exchange

Techniques:

pH gradients in the surface of lipid bilayer

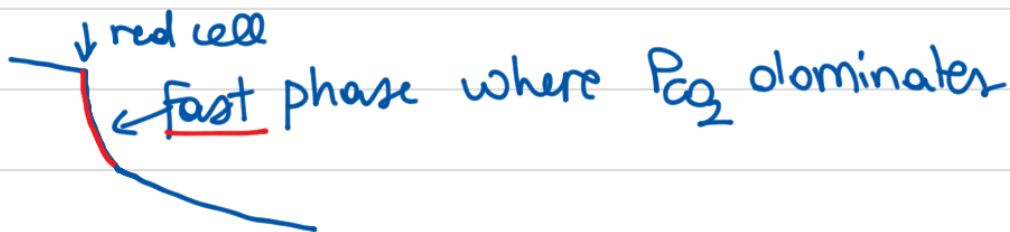
$t_{1/2}$ of CO_2 uptake $\sim 12 \text{ ms}$ (Endeward et al 2008)

In the case of CO_2 kinetics, stopped flow is not good

we have chemical eq but not isotopic equilibrium \Rightarrow take advantage of this in ^{18}O technique

$\left. \begin{array}{l} P_{\text{HCO}_3^-} \\ P_{\text{CO}_2} \\ \text{CA activity} \end{array} \right\}$ are the 3 main parameters

↓ red cell
← fast phase where P_{CO_2} dominates



monitor p_H continuously

How do extract P_{CO_2} ?

6 ODEs

Estimate P_{CO_2} , $P_{HCO_3^-}$, A_{in} , A_{out}
estimate first

fitting procedure

excellent fit

Phase 1



$t_{1/2} = 5s$ for $CO_2 \leftrightarrow HCO_3^- + H^+$

$t_{1/2} = 250s$ (isotopic exchange)

Phase 2

volume fraction of RBC is very critical ($\uparrow v \Rightarrow$ 6 time faster)

trick = ^{use} small v to reduce the time resolution for mass spectrom

$P_{CO_2} = 0.15 \text{ cm/sec}$ by RBC

Sensitivity

K_{eq} is important

A_i is very critical parameter $\Rightarrow A_i$ and p_{H_2O} need to be controlled

p_{H_2O} is also " " " "

p_{H_2O} is not critical

P_{H_2O} " "

How about ULs?

theoretical hydrodynamics

$\delta \sim \text{viscosity } \nu \times \sqrt{\text{cell diameter } l} \Rightarrow$

$\nu = 0 \Rightarrow \delta = 0$

$\uparrow \text{dextran} \Rightarrow \uparrow \delta \text{ for } CO_2$

Extrapolate to $\nu = 0$

$P_{m, CO_2} = 0.16 \text{ cm/sec}$

$P_{CO_2}^{\text{app}}$ in saline = 0.12 cm/sec $\Rightarrow \delta = 0.5 \mu\text{m}$ in saline

$P_{CO_2} = 0.15 \text{ cm/sec}$ \rightarrow 50% due to AQP1
 \searrow 50% due to Rh proteins

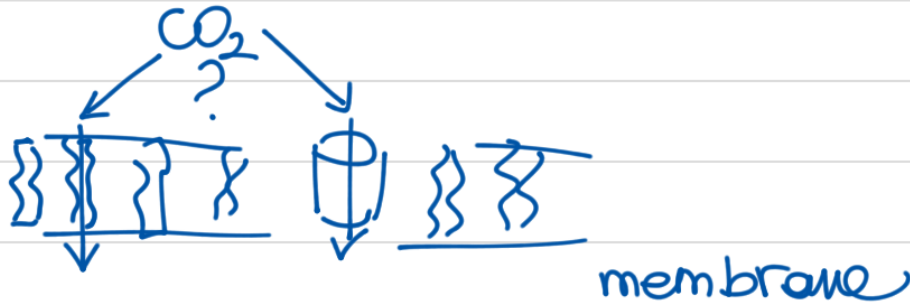
Endeward et al, 2008

2 channels

$$P_{CO_2} \approx 100 P_{HCO_3^-}$$

Lecture 4: Endward - Intrinsic CO_2 permeability of cell membrane

$P_{\text{CO}_2} = 0.015 \text{ cm/sec}$ in RBC AQP4 & Rh null



Vesicles with \neq cholesterol content



contains GAT

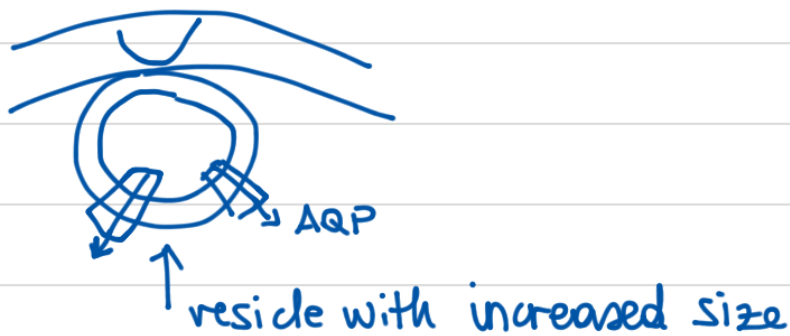
Afternoon Sessions

Lecture 5: Bhanu P. Jena - Involvement of elevated membrane cholesterol on G-protein regulated H_2O and gas transport in biological membranes



We will focus on the porosome plasma membrane

in synaptic vesicles



Jena et al 1997, PNAS

Lecture 6 : Jeff Garvin - Movement of NO across cell membr.

First described by Furchgott in 1980



Why do we care about NO? - involved in brain CNS
- mitochondrial respiration
-

NO

↑ small, non-polar
reactive

is a gas

partition coefficients are measured @ equilibrium

" " say nothing about rates

Why does the heart have AQP1? It doesn't need H₂O so why?

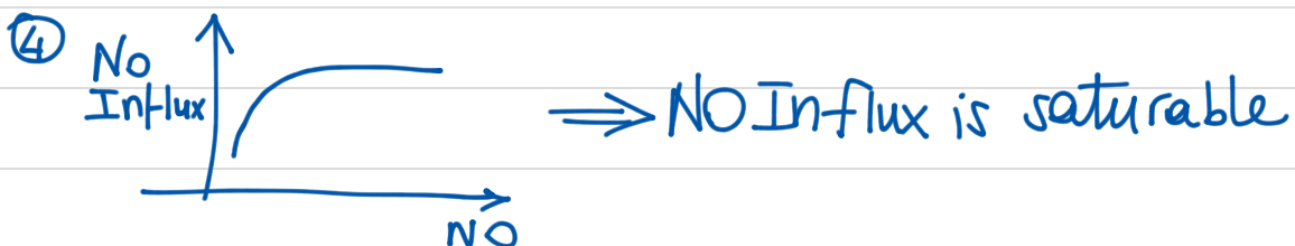
H₂O: AQP1 transports NO

Measurements: cultured cells & fluorescence

① P_{NO} correlates with P_{F}

② $\uparrow \text{AQP1} \Rightarrow \uparrow \text{NO expression}$

③ Inhibitors of AQP1 reduce NO fluxes



⑤ Purified AQP-1 increases NO transport

Conclusion:
⇒ AQP1 Transport NO

How about other AQPs?

AQP3 transports NO but not as rapidly as AQP1. Same for AQP4

Is it physiologically relevant?

Use Aortic ring preparation

Ach

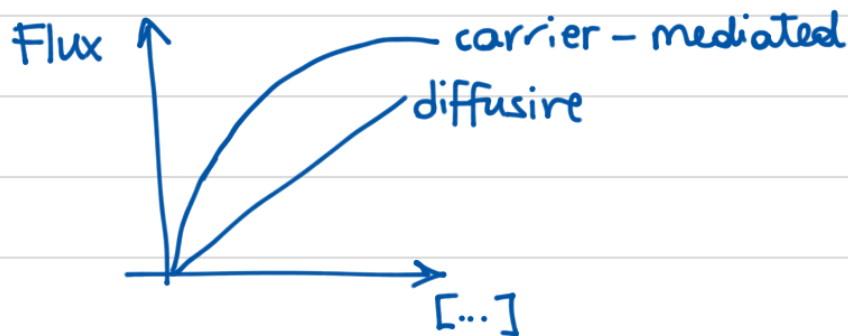
Not been able to calculate P_{NO}

Q/A:

NO electrode probably measures changes in blood flow

Lecture 7: David Weiner - Role of Rh proteins in NH_3 gas transport

Is collecting duct NH_3 diffusive or transporter-mediated?



Data show both saturable & diffusive

$$J_{\text{Tot}} = J_{\text{trans}} \underbrace{\left(\frac{[\text{MA}]}{[\text{MA}] + K_m} \right)}_{\text{saturable component}} + \underbrace{J_{\text{diff}} [\text{MA}]}_{\text{linear component}}$$

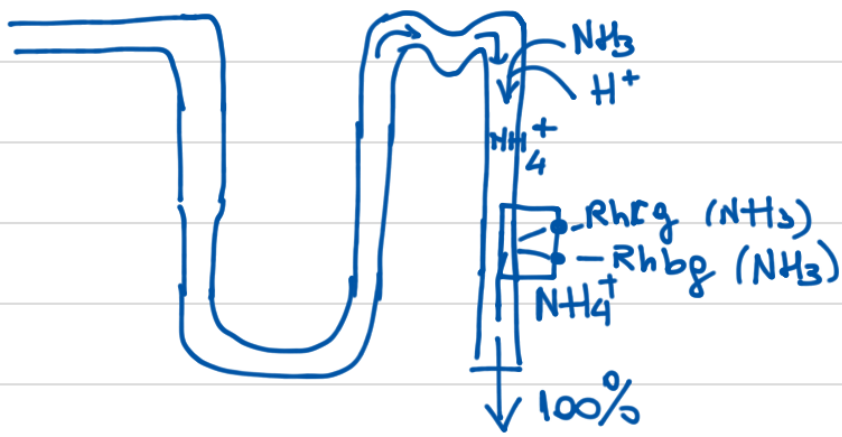
Handlogten et al, AJP Renal, 2004

Are Rh proteins present in cells with NH_3 transport?

RhAG  in RBC

RhbG in liver, kidney, sweat glands, intestine, lungs
↑
when you sweat, NH_3 ↑

RhcG in kidney, brain, testis, intestine, liver, skeletal muscle



Rh are present in cells that transport NH_3

MAc increases Rhcg expression

Rhcg & Rhbc expression increase in :

- 1) MAc
- 2) Ischemia-reperfusion injury
- 3) pt acidosis
- 4) etc ...

Keynote speaker: Robert Stroud

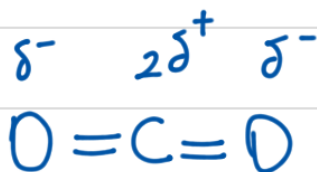
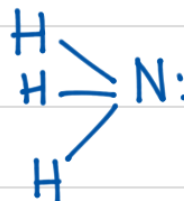
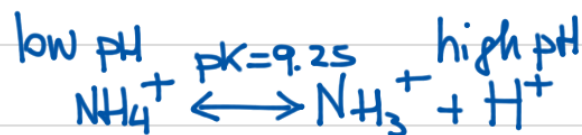
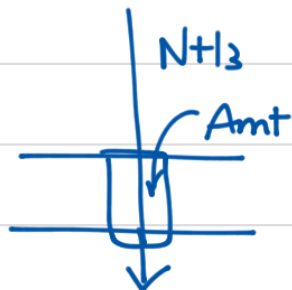
What do structures tell us about gas channels? QED!

(2) families of membrane proteins that can move gases
gases are uncharged

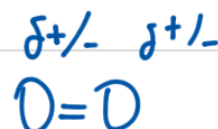
→ Rh family
AQP family

Ammonia Transport: Amt/MEP/Rh Family
in bacteria

Nitrogen Metabolism in bacteria



Dipole moment





NH_3 channel

AmtB Crystallography

↑ trimer

lyphosome

AmtB conducts NH_3 but not H_2O

Wed 28th November : <http://rmiz2012.org/> San Francisco

Day 2

09/07/12

Xue Qin : AQP5

$$\Delta p H_s^* = (\Delta p H_s)_{AQP} - (\Delta p H_s)_{H_2O \text{ control (daily matched)}}$$

T41 in AQP5

L43

No significant change in L43 mutations
Interesting changes for T41

movement of ions in the central pore
In order to see what happens we need the crystal structure

The central pore ← what is the best molecule to see what goes through the central pore

AQP6 carries very little H_2O or none
Do something to the CO_2 permeability without affecting the H_2O permeability

crystal structure → difficult

O_2 diffusion through cavities

nice packing between the helices
partition coefficient of water to octanol \rightarrow hydrophobic
channel

DIDS has no significant effect on the water permeability
in AQP5 (and probably to all AQPs)

AQP4 in astrocytic endfeet

\uparrow P_f is insensitive to DIDS

\uparrow

non specific

you get specificity by making mutations (in NBCs)
but for AQPs we do not know where the binding site
is -

glycosylation

reaction that is covalent

Wisdom : cystines within the central pore. To do : add mercury

L43C mutant: CO_2 permeability is normal

ND96 solution

reacts those cystines with other things

AQP5 has the biggest spike

DDR doesn't do anything ...

expose to a solution to be oxidized

T41C is probably misfolded

Workshop meeting - Look at future directions

① Which other families of gas channels might be there?

So far we have looked at

CO_2

NH_3

general medicine

O_2

: EPR, Optical / Hb; we want to measure fluxes of O_2 and

NO

: Hb

we want to do it faster

CO : Hb

CH_4 : swamp bacteria

H_2S : purple bacteria

N_2

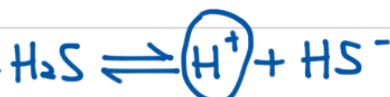
: nitrogenase - Raman Spectroscopy (fast but not sensitive)

Ethylene : plants

H_2

How do we measure N_2 fluxes?

^{13}N - NMR (not very sensitive, slow)



pH measurements

signaling gas
Optical / Hb

② What other families of gas channels might be there?

- AE1; GLUT1/4; AQP1, Rh, MCT-1

RBCs proteins

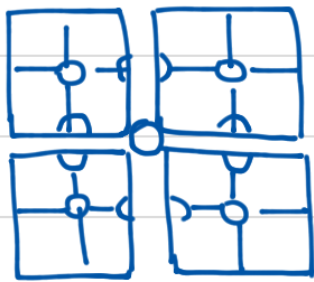
- Endothelial cells in capillaries
- BBB, BRotina-B
- BTB, BOB
- Lungs: AQPS
- Striated Muscles... myoglobin
- Mitochondrion: CO_2 is formed into the matrix - AQP8, AQP9
cytochrome oxidase

③ Physiological implications?

- Exercise
- Size scaling; Allometry: might expect to see a lot of gas channels in mice, but not in elephants
- Fish gills
- zebrafish (swim bladder)

Effects of pressure on gas permeability

Pharmacological Intervention



TETRAMER

COE experiments (Jing Lu)

NBC as a CO_2 channel

ONR global Funding Opportunities

Director's initiative : point of contact

Notes on the Discussion following Xue Qin's talk:

Clustering of AQPs: Does the gas permeability increase with clustering, suggesting an inter-tetramer contribution to permeability?

How to attack the **central-pore hypothesis**? The outermost residue is T41 in TM2. Reducing agents or Cu^{2+} ? In a T41C mutant, Cu^{2+} should bind to Cys residues and block, reversibly. Zn^{2+} or Ni^{2+} could coordinate with His in a T41H mutant. Bob Stroud thinks that we should use HgCl_2 rather than pCMBS. Worries about DIDS being non-specific. Dose-response for DIDS? Others.

General Discussion

I. What gases should we be interested in?

- CO_2
- NH_3 : general medicine. Agriculture. Microbiology (Army: Infectious diseases).
- O_2 : Optical/Hb. Phosphorescence (if fast). EPR.
- NO: Optical/Hb. Electrodes
- CO: Optical/Hb.
- N_2 : nitrogenase to turn it into NH_3 ? Raman Spectroscopy (fast but not very sensitive). Surface enhancement with gold particles? Agriculture. Microbiology. ^{13}N -NMR (not very sensitive ... talk to NMR guys)
- H_2S : Purple bacteria. H_2S electrode. $\text{H}_2\text{S} \rightleftharpoons \text{H}^+ + \text{HS}^-$... could one use pH? Hibernation gas (LaManna). Signaling gas. Optical/Hb (650-ish nm).
- CH_4 : Swamp bacteria
- Ethylene: signaling in plants
- H_2 : would it need a channel

II. What other families of gas channels might there be?

Any multimeric membrane protein whose monomers are functionally active (excludes ion channels)

- RBC proteins/ O_2 : AE1, GLUT1/4, AQP1, Rh, MCT-1 (all $\geq 100\text{k}/\text{cell}$) ... dozens of proteins 10k-25k copies/cell.
- Endothelial cells in capillaries, etc:
- BBB, BRetinaB (Pigment epithelial)
- Blood-testis barrier, blood-ovary barrier
- Lungs: AQP5 (no alveolar Rh proteins).
- Striated Muscle ... myoglobin
- Mitochondrion: MIM. CO_2 is formed in the matrix. Perhaps O_2 as well? AQP8, AQP9 (MIM). AQP5. H_2O ???
- Associations Proteomics.
- Connexins, pannexins, and similar proteins
- Strategies for finding new kinds of channels: (1) subjecting mice to chronic hypoxia and harvest RBC ... proteomic analysis. Check mRNA levels in retics. Normalize to 18S RNA, etc ... proteomics, lipidomics (\downarrow cholesterol), MCV (surface-volume ratio), P50 (pH_i , 2-3-DPG). Splice variants change?

III. What are the physiological implications of gas channels?

The gas-channel hypothesis, if true, would be a major paradigm shift ... changing the way we think about all processes involving gases. Game changer. Definitive health and performance issues. Gas channels provide:

- High flux
- Selectivity
- Control by signal transduction
- Pharmacological intervention: block or stimulate (signal-transduction: trafficking, post-translational modification) a specific pathways for specific gases, in specific places.
- Performance→Exercise, athletics, Warfighter performance, altitude: AQP1-null mouse has a 50% voluntary exercise deficit (**performance defect**). Worse at altitude. Could be due to CO₂ retention, reduced NO flux (less exercise-induced vasodilation)? Treadmill. RhAG-null. If we ever find the O₂ channel(s) ... those KOs? NO channel
- Performance→mental: AQP1-null mouse has a 50% voluntary exercise deficit. Do AQP4-null mice have ↑ cerebral capillary density to compensate for low O₂ permeability? ... but downside is susceptibility.
- Cerebral edema. Stroke, TBI, AMS ... Aeromics has a drug that blocks the aquapores of AQP4 (and AQP2) ... we hope not the gas. After the first 3 days of stroke, when edema is resolving ... stimulate AQP4
- AQP5: gas permeability of the lungs ... but downside is susceptibility. pulmonary edema. Selective drug to block P_f and stay away from gas (if it is important).
- Effect of pressure on gas permeability. In Fish ... Different channels or splice variants at different depth.
- HRE (hypoxia-response elements): which proteins unexpectedly have HREs. HIF-1 α .
- Shear stress: ↑ expression of NOS
- Are different splice variants used under different conditions?
- Size scaling, Allometry: Might expect to see a lot of gas channels in mice, but not in elephants. Also a lower O₂ consumption/gram.
- Fish gills. Compare tuna to a flounder.
- Horse has a wide range of performance.
- Joe LaManna: membranes with low intrinsic permeability—lots of proteins or cholesterol—and a high O₂ requirement, would be most likely to have gas channels. Optimizes human performance.
- Exclude gas:
- Transport gas directionally.
- Wound healing, bone-fracture healing.
- COPD: CO₂ retention,
- Stroke, MI ... low gas permeability could contribute to the development of the problem??? Increasing gas permeability could help in recovery.
- Decompression illnesses (DCS +AGE, arterial gas embolism): ↓ N₂ permeability on the way down (would also solve N₂ narcosis) ... increase it on the way up.
- O₂ toxicity:
- CO₂ narcosis:
- N₂ narcosis:
- Submarine escape ... DCI.
- Acute mountain sickness ... hypoxia
- Increase O₂ transport into tumors just before radiation
- Bacteria that need to transport gas/antibiotic. Helicobacter ...
- Parasites ... inhibit gas transport ... if the organism has a sufficiently phunky gas channel

IV. How do gases pass through the gas channel?

- Monomeric pores: AQP1 aquapores, Rh ammoniapores, UT urea pores
- Central pores (3- or 4-fold axes of symmetry)
- Side pockets (e.g., between the edges of 2 AQP tetramers)
- Corner pockets (e.g., at the corners of 4 AQP tetramers)
- Packing?: Emad tried to pack AQP4 monomers based on Fujiyoshi's/Engel's EM data. He thinks that the monomers did not get close enough together. The only reference they had. Could one do Atomic Force Microscopy (Jeff)?
- WFB/Jeff: Might it be possible to push the sides of tetramers together to see if the sides like to be together?
- Arrays: AQP4/M1 forms very small arrays, AQP4/M23 (BBB) form extended arrays of tetramers. Verkman found that it is aa17-22 in M1 that obstruct array formation. AV took MM23 Nt and transplanted it to AQP1 and got AQP1 to form arrays.
- Nanotubes, peptides that form channel-like structures ... NSF ... conduct CO₂, O₂, etc. Could be used as sensors. Cannot emphasize medical side. They fund the basic science. Plants.

V. How can we better model the movement of gases across cell membranes?

- More crystal structures
- More molecular dynamics

VI. What funding mechanisms are possible?

Early on, we have to hit at least one home run.

- ONR-BRC (Basic Research Challenge): Navy.
- ONR-MURI (Multi-Univ Research Initiative): OSD (Office of the Secretary of Defense) oversight.
- ONR-Undersea Medicine/Stress Physiology:
- ONR Young Investigator: Tenure track.
- Chief of Naval Research (CNR/2*)
- DHP (Defense Health Program): Army is the agent. Warfighter protection/performance ... AMS, ...
- PPG NIH-HL/Hypertension:
- PPG NIH-HL/Blood:
- PPG NIH-HL/Lung:
- PPG NIH-DK/: NH₃ via Rh and AQPs. Acid-base balance.
- Director's Initiative ???: Sept 25 ... no preliminary data necessary ... high risk/high impact. Up to \$5M/year. Could we get: Point of Contact. List of past recipients. Also train future scientists to carry torch. Most of winners are Associate Professor.
- ONR-G (ONR Global)/Foreign Only: VSP (Visiting Scientist Program), meetings, NICOP
- NSF: nanotubes, etc.
- Dept of Agriculture: N₂ fixation (must be done in the absence of O₂)

Ignore the following:

ACh-induced ↓ in resistance ... how affected by AQP1 KO?

WFB: We need to get together in-silico and stay in touch ... plan grant applications.

Jeff: we need to be focused ... in each grant ... stay out of KMBD (kiss of death) ... aim for Kidney Pathobiology and Urologic Diseases, Hypertension and microcirculation.

Rose:

- Please collect notes from volunteers
- PPTs
- Set up a teleconference in 1 month
- Send out these notes for annotation
-